THE UNIVERSITY OF EDINBURGH.

1. A Study of the Variations in Soil Acidity.

2. The Effect of Nitrogenous Fertilisers upon the Protein Content of Oats.

3. An Examination of the Aspergillus niger Method of Soil Analysis.

THESIS for the

Degree of DOCTOR of SCIENCE

presented by

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DECLARATION.

I DECLARE that this thesis is a record of original research work carried out since the award of the degree of Doctor of Philosophy in 1926. I have had occasional assistance in the field and in the laboratory, particularly during those periods of the growing season when an exceptionally large volume of work has had to be carried through in a short time, but, apart from a few results, which were obtained under my supervision during the year 1931-32 and have been included in fig. 5 to preserve the continuity of the observations, none of the work has previously been presented in thesis form. With these qualifications, I declare that the work has been done by myself, and that I have composed the thesis.

Preface.

The thesis is in three sections dealing with separate investigations which have been carried out, more or less concurrently, since 1928. Some results from earlier work have also been incorporated where they seem to provide suitable additional material for the questions under discussion. The three sections are connected to the extent that the same soils have been employed in each. For example, two distinct soil types occurring on the College Experimental Farm have been used for the study of soil acidity in the field and in the laboratory and for the examination of the Aspergillus biochemical method of estimating soil fertility: the investigation on oats was also conducted on one of these soils in 1933. The question of acidity, with which the first section is concerned, also comes under consideration in the other two sections and some of the statistical analyses are common to all three.

Innumerable investigations on soil acidity have been carried out in different parts of the world, but the study of the normal variation in this soil property has received little attention until three or four years ago. The problem is, therefore, a comparatively new one, and it is felt that the general technique of the method described here, of examining the effect of the plant upon the soil rather than the old established method of studying the effect of the soil upon the plant, opens up new possibilities of approach to the very difficult question of the absorption of plant nutrients. The procedure is undoubtedly artificial, but probably affords information of more direct application to practice than does culture solution work.

With respect to the second section, many investigations have been made on the metabolism of the oat plant but, to the best of the candidate's knowledge, nothing has been published on the/

the very important practical question of raising the level of the nitrogen content of the grain. The problem is quite straightforward and the conclusions are based upon what appears to be a reasonably complete set of results obtained during six seasons.

Although the subject of the third section has been creating considerable interest in the last few years, no work on Aspergillus niger seems to have appeared in this country. The possible importance of such a rapid routine test in soil analysis need not be enlarged upon at this point, and independent studies on the method are obviously required before its merits can be established. The investigation dealt with in this section is concerned chiefly with an examination of the laboratory technique and experimental error.

There has undoubtedly been a tendency in agricultural science to disregard the limitations imposed upon the data from experiments involving biological factors. The correct application of laboratory results to field practice has, of course, always been a most difficult problem, and either undue attention has been paid to analytical detail or the precise chemical method has, for convenience, been modified beyond the limits of ordinary accuracy or even replaced by a semi-quantitative test. That there exists a need for information on the reliability of the methods commonly used has been accepted in this country by the recent arrangements, promoted by the A.E.A.[1], for co-operative work on the analysis of soils and plant products; and the co-operative work of the International Society of Soil Science on methods for estimating soil fertility is another step in the direction of the proper assessment of laboratory data. There is still apparent, however, a certain reluctance to face the inevitable errors associated with all branches of work in agricultural chemistry and to employ the statistical methods that have/

[1] Agricultural Education Association

have been made available in the last few years for the examination of small populations. The significance and correlation of results can be adequately presented only on a mathematical basis: the mere tabulation and classification of figures can usually allow of only the broadest generalisations. There is now no longer any need to be content with, for example, a 2 × 2 contingency table when the machinery is available for stating the reliability of the results and so increasing their usefulness. Considerable attention has been given, in each of the sections, to this question of normal variation in the data presented but, to prevent over-emphasis, the mathematical treatment has usually been given separately in appendices.

Certain other details of technique have also been given in appendix form.

The appropriate references have been collected at the end of each section or appendix. In the case of the section on soil acidity, no attempt has been made to furnish a complete bibliography except on the particular question of seasonal variation. In the section dealing with the protein content of oats, an exhaustive search of the literature brought to light very few references to the subject under investigation. The bibliography given with the third section on Aspergillus niger is merely a selection of references bearing on particular points under discussion. The bulk of the literature on this comparatively new method has, in any case, come from the Weihenstephan laboratories, and a sufficient number of these papers has been cited to illustrate the scope of the work done there.

Most of the thesis consists of material in the following published papers to which references occur throughout the text.

1. A preliminary study of the soils of Lancashire and Cheshire. J. Min. Agric. 1929, 36, 326.

2. Investigations on Heterodera schachtii in Lancashire and Cheshire. Pt.I. (With E.G. Prentice) The errors involved in the technique. Ann. App. Biol. 1929, 16, 324.

3. Pt. II. Relationships between infestation and certain soil properties. Ibid, 340.

4. Pt. III. (With H.W. Miles.) Correlations between crop yields and infestation. Ibid, 596.

5. A capillary electrode suitable for the determination of the hydrogen-ion concentration at a point on plant tissue. (With I.M. Robertson.) J. Soc. Chem. Indus. 1930, 49, 120T.

6. A study of the hydrogen-ion concentration of the potato tuber. (With I.M. Robertson). Biochem. J. 1931, 25, 763.

7. The influence of the plant upon seasonal changes in soil acidity. (With I.M. Robertson.) J. Agric. Sci. 1931, 21, 822.

8. The estimation of the buffer capacity of acid soils. (With R. Coull.) Soil Res. 1932, 3, 10.

9. A criticism of the official method for the estimation of calcium oxide for agricultural purposes. (With A. Lauder.) Agric. Prog. 1933, 10, 172.

10. Variation in the composition of the displaced soil solution. Proc. 2nd. Intern. Cong. Soil Sci. Moscow, 1933, Comm. II, 175.

11. The estimation of the "lime-requirement" of the soil. I.S.S.S. Copenhagen, 1933, Trans. Comm. II, A, 102.

12. The variation in soil acidity. Ibid, 107.

13. The effect of additions of lime and sulphur on soil acidity, crop yield and absorption of calcium and sulphur by the plant. (With A. Robertson.) Ibid. Proc. Comm. IV.

14. Observations on the effect of various fertilisers on soil acidity. (With A. Lauder.) Agric. Prog. 1934, 11, 93.

15. The increase in the protein content of oats obtained by delaying the application of nitrogen. Scot. J. Agric. 1934, 17, No.4.

16. Available plant food in soils. New biochemical methods of estimation. (With R. Coull.) Scot. J. Agric. 1932, 15, 262.

17. A study of the technique of the Aspergillus niger method of estimating soil fertility. (With A. Dryburgh.) I.S.S.S. Copenhagen, 1933, Proc. Comm. IV.

18. The examination of soils by means of Aspergillus niger. (With A. Dryburgh.) J. Soc. Chem. Ind. 1934, 53, 250T.

Some/

Some aspect of soil acidity enters into all the papers 1 - 14, with the exception of numbers 2 and 5 which are in turn intimately connected with numbers 3 and 6. Many of these papers, however, consist merely of brief descriptions of new methods and concise summaries of results. It was, therefore, considered desirable to collect the observations bearing more particularly upon the question of the variation in soil acidity and prepare a single statement of the investigations in section I. It was also felt that a critical review of the data could not be made without a preliminary examination of the experimental errors involved and this has been incorporated, partly in the text and partly in the appendices.

The immediate practical importance of the results is regarded as the chief feature of the investigation described in section II and only one paper (15) summarising the position has been published so far. The experimental details and the statistical analysis of the different sets of results are presented here for the sake of supplying certain data connected with the other sections.

The material of section III is included in papers 16, 17, 18 but since number 17 is not available, except in mimeograph form, the results of the investigation have been rewritten.

Copies of papers 3, 4, 5, 6, 7, 8, 9 and 12 are attached since, with one exception, they contain material which has only received brief mention in the text. Numbers 2, 10, 11, 14 and 18 are reproduced in the text and appendices, while copies of numbers 13, 15 and 17 are not available.

C O N T E N T S.

A Study of the Variation in Soil Acidity.

INTRODUCTION.

Some idea of the outstanding importance attached to the question of soil acidity is readily gathered from a survey of the current literature on agricultural science, but a proper appreciation of the subject is of comparatively recent date. Even when the use of indicator solutions attracted soil investigators to measure the actual hydrogen ion concentration of soil extracts rather than the titratable acidity, the technique was not simple enough to encourage the general application of the method; and, even when the hydrogen electrode became an instrument of increasing importance in physical chemistry, its usefulness in the examination of soils was limited, by the nature of the determinations, to intensive rather than extensive studies. The great enthusiasm for soil pH determinations may be said to have followed the introduction of Bïilmann's quinhydrone electrode (6) in 1921and the realisation, soon afterwards, of the close relationship existing between the acidity and base exchange properties of the soil. The researches of Gedroiz (15), Hissink (21) and Wiegner (70) in this respect, and a comprehensive statement of the position by Page (41), probably provided the chief impetus to a renewed attack on the subject of soil absorption and the new problems associated with it.

The simplicity and speed of the quinhydrone electrode were soon appreciated by soil investigators, and there followed, from laboratories all over the world, a very large amount of data on soil acidity so that the literature on the subject increased rapidly. It was not until 1929 that certain doubts, which had been entertained for a few years, about the universal applicability of the quinhydrone electrode, were shown to be justified (19), but by that time the glass electrode had received a good deal of

attention (35, 40) and a suitable technique was soon developed for its use in soil work (38). Wherever a soil is suspected of giving erroneous results with quinhydrone, on account of the presence of manganese dioxide, the investigator can turn with confidence to the glass electrode. The machinery is, therefore, available for the rapid and accurate determination of soil acidity in presence of water.

It is possible, however, that the ease with which pH determinations can be made has been instrumental in obscuring their real meaning. There is no necessity to describe the value of such measurements, but there has undoubtedly been a tendency to overrate their importance. The pH value of a soil is merely a characteristic of the particular sample at a particular time and, unless it is so regarded and considered in conjunction with other properties of the soil, there is a real danger of misjudging or misinterpreting its significance. It is to be feared, therefore, that a large number of the published figures on soil acidity have been collected without due consideration of their relative and absolute values. Correlations between pH figures and other dependent and independent variables have also been a feature of publications during the last ten years, but their validity is frequently very largely discounted by an unconscious or studied neglect of attention to the actual meaning and limitations of the figure for soil pH.

In the course of the collection of data on soil properties and on the relationships existing between soil and plant, the writer has been impressed by the manner in which the hydrogen ion concentration of a soil may change, and has felt that this very important behaviour of certain soils has not received adequate consideration. Apart from a single paper in 1923 by Kelley (27), showing the variation in soil acidity during

a/

a year, the question seems to have attracted little attention. Since the publication, in 1931, of the results from some preliminary experiments by Smith and Robsertson (57), several investigators have brought forward additional evidence on the seasonal variation in soil pH, but the data have not been supplemented to any extent by other experimental results and the reasons or causes assigned to the variation have been largely speculative. There is no doubt that the growing plant plays an important part in the phenomenon, and the material collected during the past six or seven years on this subject is presented in the following pages.

An attempt is first made to assess the errors associated with soil analysis in the laboratory: that is followed by an examination of the errors experienced in pot experiments and the greater errors incurred in the field. The results obtained in the course of several investigations carried out under field and laboratory conditions are then submitted and discussed in relation to the mutual effects of soil and plant. The results are generally summarised in graph form and included in the appropriate section, whilst experimental details, calculations and supplementary work are given in the appendices.

EXPERIMENTAL ERROR IN SOIL ANALYSIS.

The question of experimental error does not ordinarily excite much attention in chemical analysis when one is dealing with relatively small quantities of pure substances. The materials are homogeneous or can be ground to a state of very fine division so that sampling errors are very small: the errors involved in the subsequent analysis may readily be checked at various stages in the procedure, and the total error permissible,

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by the generally accepted methods, is commonly less than one per cent.

In the analyses of soils or plants, the state of affairs is quite different. The material, in the first place, is not homogeneous; in the case of soils, grinding introduces complications except when a complete analysis is to be carried out; in the case of plant material, grinding does not usually produce an impalpable powder. The sampling errors in the laboratory are, therefore, of considerable importance.

1. Laboratory Error. The almost universal practice in preparing a soil in the laboratory for analysis is to break up the lumps by means of a wooden pestle or roller and remove the stones by means of a 2 mm. sieve. When only a small quantity of this "fine earth" portion can be examined, as, for example, in the determination of carbon or nitrogen, it is necessary to grind a representative sample to a fine powder from which small amounts can be taken without incurring serious sampling errors. The errors of the determination may then be regarded as due mainly to the analytical technique. The same may be said to be the case when the clay fraction is separated for special examination. The experimental errors in such cases really fall into the category of errors of chemical analysis where the sampling errors are small.

For other determinations, where larger samples require to be used, it is not only impracticable to grind a large quantity but it is not permissible to employ the ground sample. For example, it has been shown that both the amount and relative proportions of the exchangeable bases in a soil are seriously affected by grinding the silicates (28). It is necessary, therefore, to take samples from a bulk sample of a heterogeneous mixture/

mixture of particles, varying not only in size, from 2 mm. down to colloidal dimensions, but also in constitution. The largest particles may, for example, be solid particles of mineral matter or they may be aggregates of colloidal material, and the relative proportions of each must obviously depend upon the character of the soil under examination. That question was simply tested by estimating the exchangeable calcium of the two fractions of a 3 mm. sample of soil separated by a 1 mm. sieve (51).

Milligram equivalents of exchangeable calcium per 100 g. air dried soil.

	Fraction	
Soil	under 1 mm.	over 1 mm.
170	9.43	9.68
277	7.26	8.06

In each case, there was more exchangeable calcium in the coarse fraction than in the fine fraction due, either to the small amount of fine gravel present, or to the relatively large amount of exchangeable calcium in the binding material of the aggregate greater than 1 mm. in diameter. It is obvious, therefore, that such a mixture as a soil must be sampled with extreme care in order that the errors incurred in taking the sample should not invalidate the care demanded in the subsequent analytical procedure. In spite of careful laboratory sampling, however, it is probable that the errors due to sampling outstrip those of analytical technique, and it is instructive to examine a few cases before proceeding with the question of soil acidity.

Robinson and Lloyd (48) gave some attention to the subject in the analysis of soils for survey purposes. They found that the field error was very large in comparison with the laboratory error, and that in a mechanical analysis or total phosphate determination the probable laboratory error was about

2.5/

2.5 per cent. - a standard error of 3.8 per cent. There is not much doubt that a larger proportion of this error lay in the laboratory sampling than in the subsequent operations. The technique employed in a mechanical analysis or in a total phosphate determination is rather more complicated and subject to error, however, than a large number of simpler measurements commonly made with soils. It might reasonably be expected, for example, that the analyses of various soil extracts in water, prepared under controlled conditions, would provide a good estimate of the error due to the preliminary sampling of the soil, and the following figures have been obtained in the collection of the data reported on pp. 45.

(a) Soil extracts. In this case, a large amount of air dry soil was thoroughly mixed, by repeated sieving, and divided in 100 g. portions. These were kept moist for different lengths of time at a temperature of 18-20°C. and were then shaken with 500 c.c. water for 30 minutes in an end-over-end shaker. A clear aqueous extract was then obtained by filtration and calcium was determined in a definite volume of each extract. Each test was carried out with duplicate portions of soil so that an examination of the duplicate determinations gives an idea of the degree of variability to be expected in such an analysis. The determination of a small amount of calcium (the figures never exceeded 1.7 mgm. equiv. per 100 g. soil) in a soil extract is obviously subject to greater errors than would be incurred in a straightforward chemical analysis, but it is fairly typical of much soil work. The statistical examination is given on p. 65 and shows that a difference of 0.05 mgm. equiv. per 100 g. soil, equal to about 7 per cent., would be unusual in duplicate determinations by this method.

(b)/

(b) Eelworm infestation. The results of another investigation, on a totally different subject, seem to throw a good deal of light upon this question of the laboratory error of sampling a soil. One method of estimating the infestation of an area suffering from "eelworm disease" is to count the encysted females of Heterodera schachtii in the soil, and a study was made of the errors involved in the technique (56). The number of cysts in 10 c.c. soil varied from about 10 to 50 or 60 in heavily infested areas, and it was demonstrated that the numbers of cysts found in soil samples, taken at random from a bulk sample, were distributed according to a Poisson series (see pp. 66-71). Although the standard error due to the laboratory technique was of the order 6 per cent., the mean value of 10 counts was actually a reliable estimate of the number of cysts, and, even if the technique had been perfect, there would still have been an inherent percentage standard error comparable to that mentioned. Now in this case the laboratory error lay almost entirely in the taking of the individual samples of soil from the bulk sample, the subsequent procedure being merely the shaking up of the sample with water and the counting of the cysts, which floated to the surface, under a low power lens. The distribution of the cysts in the original bulk sample must have been comparable to that of soil particles of a similar size, and the errors involved in sampling for cysts counts would seem to be closely related to those in sampling for other purposes and especially for mechanical analysis. As a matter of fact, Robinson and Lloyd (48) found that the probable laboratory error in mechanical analysis varied considerably for the different soil fractions and that it was about 6.3 per cent. in the case of fine gravel. This is equivalent to a standard error of between 3 and 4 per cent. for the mean of 10 analyses and is rather less than the/

the figure for cyst counts. One point which merits attention, however, is that the soils concerned in this investigation were of a peaty character and were, therefore, more difficult to handle than normal mineral soils.

(c) Biochemical analysis. There are certain other branches of soil investigation in which the errors of laboratory technique are due not only to the preliminary sampling of the soil but to biological factors which are not always under control. For example, in the dish experiments reported on pp. 38-45, the experiments were invariably carried out in duplicate, but it was impossible to ensure the same rate of growth of the plants in duplicate dishes. A great variety of factors is obviously involved in such a case and it is only after frequent repetition of the experiment that the results can be regarded with confidence. The same applies to the biological or biochemical methods of estimating the manurial requirements of soils, and an example of the errors associated with the Aspergillus niger method is given in section III, pp. 150-156. It is shown that, in the determination of available potassium or phosphorus by this method, the standard error of the mean of 10 replicate tests was 5.68 and the percentage standard error 4.5 to 5.0 for an individual determination. Such accuracy may be regarded as quite good enough for this type of routine test, for it is in the interpretation of the results that the chief difficulty arises.

(d) pH measurements. With respect to the errors in the measurement of the hydrogen ion concentration of soils, a good deal of recent co-operative work has been carried through so that both the reliability and accuracy of different methods are now well known. The literature on the subject is very extensive, but attention may be directed to the report of the Soil Reaction Committee/

Committee of the International Society of Soil Science (60) and to the more confined work of the Soil Analysis Committee of the Agricultural Education Association in this country (4). When a technique, such as that recommended for the quinhydrone electrode by the I.S.S.S. committee, is followed, the agreement obtained by different workers is quite satisfactory provided, of course, that the soils are free from substances which react with the quinhydrone. When the potential of the electrode exhibits a drift, in the first few seconds after the mixing of quinhydrone with the soil, the measurement is not satisfactory and only an estimate of the true pH can be obtained by taking a reading at once. In such cases, it is necessary to employ a colorimetric method or a glass electrode, and it is probable that the latter will become increasingly popular. It possesses the great advantagesthat (1) glass is the only "foreign" substance introduced into the suspension, (2)a true measure of the hydrogen ion concentration is obtained even in presence of strong oxidising and reducing agents, (3) the potential is established almost instantly and (4) a working range of pH 1.5 to 10.0 is great enough for most purposes in soil work. The disadvantages of working with a fragile glass membrane of very high resistances have been largely overcome by improvements in the design of the electrode and by the use of a potentiometer circuit incorporating one or more thermionic valves to amplify the current. When a soil is free from the active form of manganese dioxide, which appears to be responsible for secondary reactions with quinhydrone, the glass electrode does not possess any advantage over the quinhydrone electrode. None of the soils dealt with in this work showed any appreciable potential drift with the quinhydrone electrode, which has been used throughout, with a Cambridge potentiometer unit, essentially as recommended by the Soil Reaction Committee (60).

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With respect to the accuracy of the measurement, it is obvious that any one worker may be expected to obtain more uniform results than a series of workers, and the variation in the pH of duplicate samples is usually small. A few examples are given below to illustrate this point. In the series of duplicate samples which were used in preparing aqueous extracts for calcium determinations (see above), portions of the suspension were taken for pH measurements. The greatest difference in potential between duplicates was 3 mv. which corresponds to about 0.05 pH unit, while the average difference was between 1.3 and 1.4 mv. These are values which are quite typical of many hundreds of duplicate pH determinations which have been made in the course of recent years. Other examples may be taken from the dish experiments reported later (pp. 38-45). In the incubation experiments with potatoes, two small samples of soil were taken for pH measurement from each dish, which originally contained 500 g. soil; the dishes were also in duplicate. A set of 50 pairs of results from individual dishes, taken at random from the tables, shows that the standard deviation of the difference between duplicates was 2.1 mv. A similar set of 50 observations for duplicate dishes had, as might be expected, a rather greater deviation of 5.6 mv. In the experiments with peas, oats and barley, the seeds were planted in about 15 g. soil and the whole sample was taken for the pH measurement. In this case a set of 50 results from duplicate dishes gave a standard deviation of 2.4 mv. while a similar set from unplanted control duplicate dishes gave approximately the same value, viz. 2.6 mv.

On account of the presence of plants in many of these cases the above results are higher than would normally be found for dry soil samples, but they show that with a standardised technique and suitable care in sampling, the variation to be expected/

expected in the pH value of duplicate samples of soil in laboratory experiments is only of the order 0.05 unit. In terms of hydrogen ion concentration, such a variation is, of course, considerable, being rather more than 10 per cent., but, in reality, it is quite small compared with the normal variations encountered in soil acidity to be described later. When a sample of soil contains particles of free lime or calcium carbonate, it is sometimes difficult to obtain constant and uniform pH values, but this may be regarded as a particular case in which an accurate pH measurement is not of much importance.

From the above observations on the results obtained in different lines of work, it appears that the errors associated with the laboratory technique in soil analysis may be as great as 10 per cent. and that in the determination of the hydrogen ion concentrations of soil suspensions that figure serves as a useful guide in the interpretation of small differences.

2. The Error in Pot Experiments. Before going on to examine the question of the normal variation found in field experiments, it is desirable to give some attention to the errors incurred in pot experiments. On account of the fact that several pots are commonly filled from a large composite sample of soil from the field, which has been thoroughly mixed and riddled, the pot experiment is bound to be capable of yielding more accurate results than a plot experiment with the same soil.

The soil is only one factor affecting crop yield so that data on the yields from individual pots can scarcely be accepted as a sure measure of the error of the original sampling. An indication of the variation is given, however, in the results from the following experiment. A large composite sample of soil weighing about 1000 lb. was broken up, passed through a $\frac{1}{4}$" riddle and/

and divided into three portions. Portion 1 received the addition of 0.02 per cent. sulphur, portion 3 received 0.1 per cent. calcium hydroxide, while portion 2 was left untreated. Every precaution was taken to ensure that the sulphur and calcium hydroxide were uniformly incorporated in the soil. Each portion was then subdivided into 10 samples which were placed in 12" pots. Potatoes were planted two days later and the pots watered. A sample of soil was taken from each pot by means of a small auger three days after planting. The sulphur and calcium hydroxide had only been in contact with the soil for five days and the effect of the sulphur was not apparent in the first week, but the results for each set of ten pots were examined separately. The first plants through the surface did not appear for some three weeks so that their influence could be neglected. The standard deviations were 2.2, 1.4 and 3.7 mv. respectively for the sulphur, untreated and lime sets. In other words, the degree of variability amongst 10 pots filled from the same sample of soil was of the order .04 or .05 pH unit, which is similar to that found for laboratory sampling.

These pots were sampled at frequent intervals and the first 40 sets of pH figures for duplicate unplanted pots were examined to estimate the variability between pots over 3 growing seasons. The standard deviation of the difference between duplicate pots was 5.3 mv. in the case of the sulphur-treated soil, 3.1 mv. in the case of the untreated soil and 3.0 mv. in the case of the lime-treated soil.

These larger variations were not due entirely to sampling because the hydrogen ion concentration of the soil fluctuated considerably during the above period of observations and the fluctuations were naturally greatest in the case of the soil which was treated annually with sulphur. The relationship which/

which necessarily exists between acidity and other soil properties, however, suggests that the figures given above furnish a reasonable guide to the errors associated with the sampling of a soil in pot experiments. In this instance, they may be taken to mean that a difference of more than 0.1 pH unit between two samples is unlikely to be due to sampling error.

The results obtained in pot experiments are usually recognised to be not directly applicable in the field on account of the rather special treatment given to the plants and the absence of subsoil effects. It does not seem to be so generally recognised that the mere handling and preparation of the soil before potting exerts an enormous influence on its properties by producing much more rapid changes than could occur in the field. When a soil is well aerated and kept at a moisture content suitable for plant growth, there is a rapid increase in the concentration of the soil solution (54). The details which are given (Appendix III) show that the concentrations of calcium and magnesium in the displaced soil solution may, in fact, be doubled in the course of a month's storage and practically mask the effects of addition of limestone to a soil. Differences which might be observed under field conditions are liable, therefore, to be obscured or exaggerated in pot experiments as a result of the intensive biological activity which occurs, to begin with, under the more favourable moisture and air conditions. The relative accuracy of the pot experiment is, therefore, misleading and is sometime responsible for erroneous estimates of the value of the results.

3. The Error in Field Experiments. In the field, the sampling difficulties are naturally much greater on account of the comparatively large fluctuations which are possible in the properties/

properties of the soil, and these difficulties are increased by the many biological and climatic factors outside experimental control. The consequence is that, even in agricultural chemistry, the accuracy attainable in the laboratory is in an entirely different category from that possible in securing the material for analysis.

More than 30 years ago, Leather (31) drew attention to the question of variation in soil samples; and about 25 years ago, Wood and Stratton (71) and Mercer and Hall (37) emphasised the need for caution in interpreting experimentsl results and showed how normal variations in composition and yield of crop might be dealt with. As in pot experiments, the soil is only one of the factors concerned in the production of a crop, so that figures for crop yield on different plots do not provide a means of estimating the errors incurred in the sampling of the soil. At the same time, the results cited by the above investigators, and the numerous more recent data from uniformity trials, suggest that the soil must vary considerably from point to point over an apparently fairly uniform area. Robinson and Lloyd (48), in their study of this question, came to the conclusion that the field error was about twice the laboratory error in mechanical analysis, and much more than that in chemical analysis. Figures of ± 5 and ± 10 per cent. respectively were suggested as liberal estimates of the probable field errors for mechanical and chemical analysis associated with the sampling of a soil on a reasonably uniform area.

These figures bear a striking similarity to the errors associated with the estimation of the infestation of H. schachtii and discussed in Appendix II. In this case, the question involved the degree of variability in infestation over small areas and the total error due to field sampling and laboratory technique/

technique was large, but the field error was about twice the laboratory error.

Although there has been an increasing effort in the last few years, particularly in this country, to carry out field experiments in such a way as to secure suitable estimates of the variability due to casual factors such as the soil, very little seems to have been published concerning the normal variations in the properties of the soil over small areas. Post (42) made an examination of the soil variability on small plots in so far as nitrogen content is concerned and found that the laboratory error was so low compared to field error that it could almost be disregarded: but, generally speaking, this point has been seriously overlooked, and many investigations have unfortunately been carried out with great attention to analytical detail but without due appreciation of the preliminary sampling errors.

(a) General observations. There is no doubt that differences in soil properties are frequently revealed in the field by rather obvious differences in crop or flora. For example, the incidence of leaf stripe on oats is commonly observed to be associated with soil acidity and the following pH figures are quite typical:-

Field	A	B	C
Area affected with stripe	7.46	6.93	7.72
Adjoining area, unaffected	5.61	5.90	6.43

Such results may be obtained over areas which have apparently had the same history of cropping and treatment. Similar observations may be made on grazing land where the plant associations sometimes vary to a considerable extent from point to point on a field. The following pH figures were obtained for samples of soil taken from clover patches and practically clover-less patches a few inches apart:-

Field	A	B	C	D	E
Clover patch	4.97	5.35	5.20	6.22	5.68
Cloverless patch	4.62	4.95	4.50	5.82	5.09

It is quite obvious, therefore, that soil acidity may vary between fairly wide limits within very small distances on a field. It is also evident, of course, that the nature of the herbage is not determined directly by the pH figure, but this question will be discussed later.

(b) Normal variation in acidity. Quite apart from differences revealed by plants, however, innumerable individual borings or composite samples have demonstrated that a considerable variation in soil acidity may exist from point to point over even quite restricted areas. Headland influences are well known, but surprisingly large differences in hydrogen ion concentration may also be found along strips or through plots which, from all outward appearances, are perfectly uniform. A few examples will suffice to illustrate this point.

In the investigation on H. schachtii previously mentioned, an attempt was made to discover whether any correlation existed between the pH values and the cyst counts for various samples (53). As described on pp. 66-72 , a narrow strip running across a portion of a field was divided into lengths giving a series of ten plots each about 50 to 75 sq. yards in area, and a composite sample of soil consisting of at least 10 borings was taken from each plot. A statistical analysis of the pH figures, for the series 23-32 and 66-75 of peaty sands and for the series 46-55 and 11-20 of peats, shows that the standard deviation is equal to 0.163 or, in other words, the odds are even against a difference of about 0.15 pH unit between two plots being due to anything but chance. Consequently, in strips about 100 to 150 yards long running across these particular fields, only differences in/

in acidity amounting to about 0.4 pH unit could be regarded with reasonable certainty as not being due to chance. A change in pH value from, say, 6.0 to 6.5 is equivalent to a decrease in hydrogen ion concentration of nearly 70 per cent. which gives some idea of remarkable variations in the degree of acidity which may occur in the field.

In a more recent experiment in connection with the improvement of a lawn by manurial treatments, an area of 5,600 sq. feet was divided up into 8 randomised blocks of 7 plots, each of which was 10 feet square. A composite soil sample of 9 borings was taken from each of the 8 control plots four times during the period March to August. An examination of the pH figures for each set of results for these plots showed that, even over such a small specially prepared area, the standard deviation was 0.122 pH unit for any plot or 0.173 for the difference between two plots. This is rather more than 3 times the laboratory error and shows that the field error is approximately 0.166.

A similar result was obtained from a plot experiment described later, p. 27 . In this case 4 potato plants on an untreated plot of 220 sq. feet were marked and a sample of soil was taken from the roots of each plant 7 times during the growing season. The standard deviation of the difference between samples from pairs of plants was 0.17 pH unit. In this experiment, the normal variation in the soil might be said to have been reduced to a minimum by taking the samples always from the same spot and that the variability was due largely to the influence of the growing plant. The result demonstrates, however, the order of the differences to be expected between duplicate samples of soil from a plot experiment during the growing season and the magnitude of the field sampling error.

It/

It is now possible to assess with some degree of precision the seasonal fluctuations in soil acidity which have been observed in the course of a study of the question during 5 years and which may be regarded as lying outside experimental control.

SEASONAL VARIATION IN SOIL ACIDITY.

As previously mentioned, the hydrogen ion concentration of a soil cannot be regarded as more than a single characteristic of the soil under a particular set of conditions, and it has been known for a long time that it is markedly affected by the presence of salts even in small amounts (16). Since the concentration of electrolytes in the soil may show considerable seasonal variation, it is natural to look upon fluctuations in soil acidity as being intimately connected with changes in the soil solution.

The pH values of the soil have been found to change regularly in some cases, and merely to fluctuate about a certain value during a considerable length of time in other cases. For example, Kelley (27) made monthly tests for a year and found that, although the pH value might vary as much as one unit during the year with some soils, the changes were generally small. Usually, but not invariably, the acidity increased during the dry summer months, or on freezing, and decreased to the original value with rain or thaw . There was, however, no apparent relationship between the original pH value and the observed variations. Baver (5) came to the conclusion that with acid soils the pH value decreases during summer, but that with approximately neutral soils the changes are irregular. In a more recent paper, Swanback and Morgan (64) report the changes found during three years. The crop was tobacco, and the pH values reached a minimum during summer

and/

and returned to approximately the original figures during the following spring.

A number of still more recent papers, published since the present work was started, provide sufficient data to show that the changes in soil acidity cannot be simply expressed. Harris (17), for example, foundgreat irregularities under all conditions of cropping, the fluctuations over a short period in summer being sometimes as great as the total annual variations; he also observed that the seasonal fluctuations were not constant. Hester and Shelton (20), also in the United States, found that low pH values in July and high values in December corresponded respectively with high and low concentrations of water soluble constituents in the soil. In Hungary, Fehér has also found an inverse relationship between pH value and electrical conductivity, although his minimum pH values occurred in autumn (12). Salminen (50), in Finland, has discussed the changes in relation to the drying and wetting of the soil, while Radu (44) has examined the changes in relation to the temperature and moisture conditions and the colloidal complex of the soil. There is no doubt, therefore, that the question is attracting the attention of soil workers in different parts of the world and that discrepancies in different sets of results are to be attributed mainly to diverse local conditions of climate and soil.

In the course of an intensive investigation of a large number of potato plants in 1929, the soil beneatheach plant was sampled several times during the growing season. A study of about 500 pH determinations of these samples revealed interesting fluctuations, similar to those mentioned above, which suggested a close correlation between soil acidity and stage of plant growth. In order to examine this point more closely a series of field and plot experiments was carried out in 1930, and the plot experiments/

experiments have been continued until 1934. Various pot and incubation experiments have been carried out concurrently in order to test the field results with greater facility and precision. Brief notes on all the soils investigated may be most conveniently given at this stage.

1. The Soils Examined. Although all the soils occur in a comparatively limited area of about 6 miles in radius in the neighbourhood of Edinburgh, there is a considerable variation in their chief characteristics.

Soil B. College Experimental Farm, Boghall, elevation 600 feet. Most attention has been given to this soil. It has been studied in a series of field plots and in pot and incubation experiments. The mechanical analysis by the International Pipette Method (3) gives the following percentage composition:- coarse sand 20, fine sand 26, silt 24, clay 25, air dry moisture 3.7, loss on ignition 7.4. The "Lime-requirement" by the Hutchinson and MacLennan method (25) is about 0.20% $CaCO_3$.

Soil P. Boghall, elevation about 1300 feet. The sample was taken from a peaty layer 9-10 inches deep overlying well weathered basic andesite. This peaty soil has been examined only in incubation experiments. The loss on ignition of the oven dry material is 48 per cent.

Soil W. Boghall, 600 feet. This is a light sandy soil taken from a mound of glacial sand, which at one time supported trees but which was cleared a few years ago and has not been cultivated during the last 70 or 80 years. The loss on ignition, due largely to partially decayed organic matter, is 9 per cent., whilst coarse sand amounts to 50 per cent., and fine sand to 20 per cent. A previous investigation of this soil (51) showed that 100 g. air dry soil contained only 5.6 mg. equivalents of exchangeable/

able bases, of which nearly 70 per cent. was iron and aluminium.

Soil G. This is a sandy soil, from an uncultivated raised beach close to the south shore of the Firth of Forth, and has 68 per cent. coarse sand, 10 per cent. fine sand, 8.5 per cent. loss on ignition.

Soils L, D and E are all about 100 feet above sea level and might be described as garden soils which have received large dressings of organic manures during many years of intensive cultivation. Whilst D1 and D2 represent two sections of the same field, E1 and E2 are from different fields on the same farm.

Soils C1 and C2, from different fields of the same farm, are both heavy loams.

Soil F is a soil very similar to B in regard to texture and environment at 600 feet above sea level.

Soils H and I, from different fields of the same farm, are loams and rather similar in their chief physical properties.

As far as the field experiments were concerned, the manurial treatment was essentially the same in all cases, amounting to about 20 tons per acre of farmyard manure, and 8-10 cwts. of artificial fertilisers in the usual proportions adopted for potatoes in this area. No farmyard manure or artificial fertilisers were applied in the prelimary series of plot experiments in 1929 nor to the second series, also on soil B, until 1933 when the plots were subdivided for a top dressing experiment with ammonium sulphate (see p. 107). Both series of experiments, however, involved the following treatments with sulphur and calcium hydroxide to obtain varying degrees of acidity on the same soil.

(a) Plot experiments/

(a) Plot experiments. (i) 1929 Series. Soil B. Treatment 4 weeks before planting:-

Plot 1 B2 1000 lb. sulphur per acre.
" 1 B3 untreated.
" 1 B4 72 cwt. calcium hydroxide per acre.
" 1 B5 192 " " " " "

There were no fallow plots and the average results from four potato plants on each plot are submitted (fig. 1) along with the other field observations for 1929.

(ii) 1930-1934 Series. Soil B. The area was divided into 2 sets of five 1/200 acre plots each. The treatments were:-

Set	Plot	27. 11. 29.	3. 4. 30	31. 3. 31	18. 3. 32
A	1	-	6 lb. S	-	4 lb. S
A	2	-	2 lb. S	-	2 lb. S
A	3	-	-	-	-
A	4	12 lb. $Ca(OH)_2$	-	-	-
A	5	36 lb. "	-	-	-
B	1	-	6 lb. S	4 lb. S	2 lb. S
B	2	-	2 lb. S	2 lb. S	1 lb. S
B	3	-	-	-	-
B	4	7 lb. $Ca(OH)_2$	-	-	-
B	5	21 lb. "	-	-	-

The cropping has been different on each set of plots and is shown on fig. 5. During seasons 1930 and 1931 a central strip 3 feet wide running through B set of plots was kept fallow. At regular intervals in 1930 a composite sample of 9 or 10 borings was taken from each of the plots of set A; in each of the plots in set B, a composite sample of soil was taken from the roots of 4 marked potato plants.

(b) Field experiments. 1929 and 1930. Various soils under potatoes. Twelve healthy potato plants were marked at L, C and D and two at E. In 1929, additional data were collected from four plants at F and two at H and I. Soil samples were taken from the roots of each plant at intervals between the early stages of growth and harvest and all the results for each soil at each sampling have been averaged and are presented in figs. 1 and

2/

2. Each of the lines L, C and D, therefore, represent an average for 12 samples, F an average for 4 samples and E, H and I for 2 samples. Only results for soils under crop were obtained in 1929, but in 1930 it was possible to obtain figures for fallow areas on soils C and E.

2. Determination of Acidity. In 1929, the soil samples were allowed to dry at room temperature (15-20°C.) and the hydrogen ion concentration was determined by means of the quinhydrone electrode (7). About 10 g. soil, 25 c.c. of boiled distilled water and a few decigrams of quinhydrone were shaken together vigorously for 30 seconds and allowed to stand for a few minutes before the potentiometer reading was taken. During 1930 the soils were usually examined soon after sampling and invariably within 24 hours, and the same can be said of all the pot and incubation experiments. After 1930, the samples from the plot experiments were usually allowed to reach an air-dry condition and sieved before making the pH determinations.

Aarnio (1) has described remarkable increases in soil pH by drying at 25°C. but Teräsvuor (65) found that air drying generally produced no effect, and Deines and Kleinschmidt (11) have shown that soils dried in vacuum (30°/25 mm.) suffer no pH change. A series of determinations, of which the figures in the Table below are typical, made immediately after sampling and again with the same soils in an air dry condition some months later, indicated that changes due to drying at room temperature were practically negligible.

TABLE ..

pH values before and after drying.

Soil	B1	B2	B4	B5
Freshly sampled	4.71	5.57	6.65	5.66
Air dry	4.72	5.60	6.63	5.63

As previously mentioned, it has been established (19) that errors in pH determinations by means of quinhydrone may occur with certain soils, but a number of "10 second values", as recommended by the special committee on soil reaction measurements of the I.S.S.S. (60), showed that there was no drift due to the presence of manganese dioxide in any of the soils under investigation.

3. Results.

(a) Field experiments 1929 and 1930. The first samples were not taken until the end of June, when the crop was fairly well advanced. A consideration of figs. 4 or 5 shows that a large increase in acidity had taken place by that date at a locality which is much "later" than all others with the exception of F. Consequently, the first results recorded for the field soils are probably not far removed from the minimum pH values for the season. The curves for C2 and E2 in fig. 2 suggest that that is the case. With soil C2 the greatest difference between fallow and planted soils occurred about the 20th July: with soil E2 it did not occur until the beginning of August, but the potatoes were not planted until the beginning of July and growth was naturally very rapid.

It may be assumed, therefore, that the results plotted in figs. 1 and 2 show the fluctuations only in the later part of the growing season when, generally speaking, the reaction of the soil is tending to become less acid. It will be observed that the plant exerts a modifying influence upon change in acidity for the fluctuations found for fallow conditions, which seem to be related to temperature and moisture, are not so marked in the presence of a crop.

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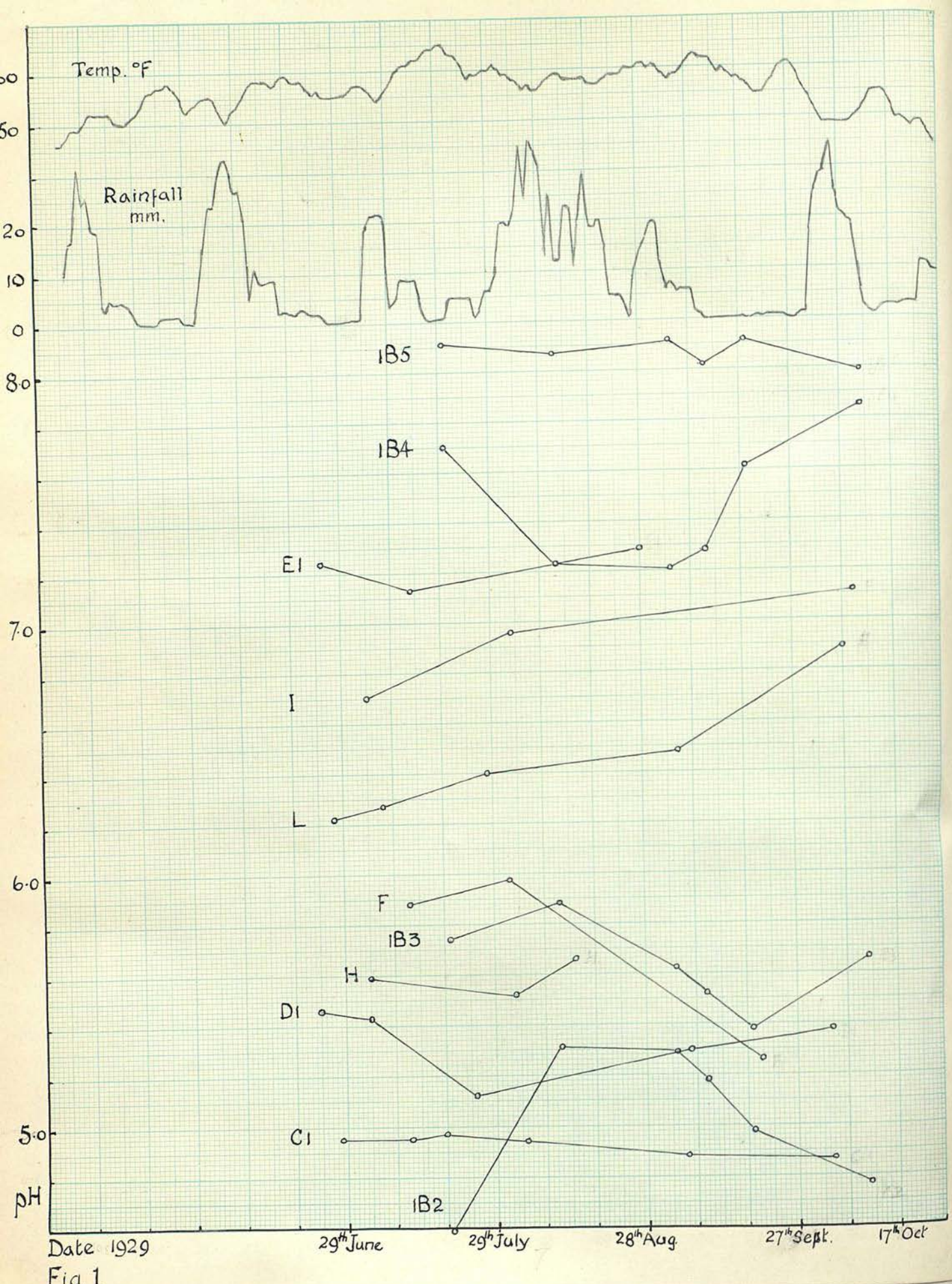
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1B5
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I
L
F
1B3
H
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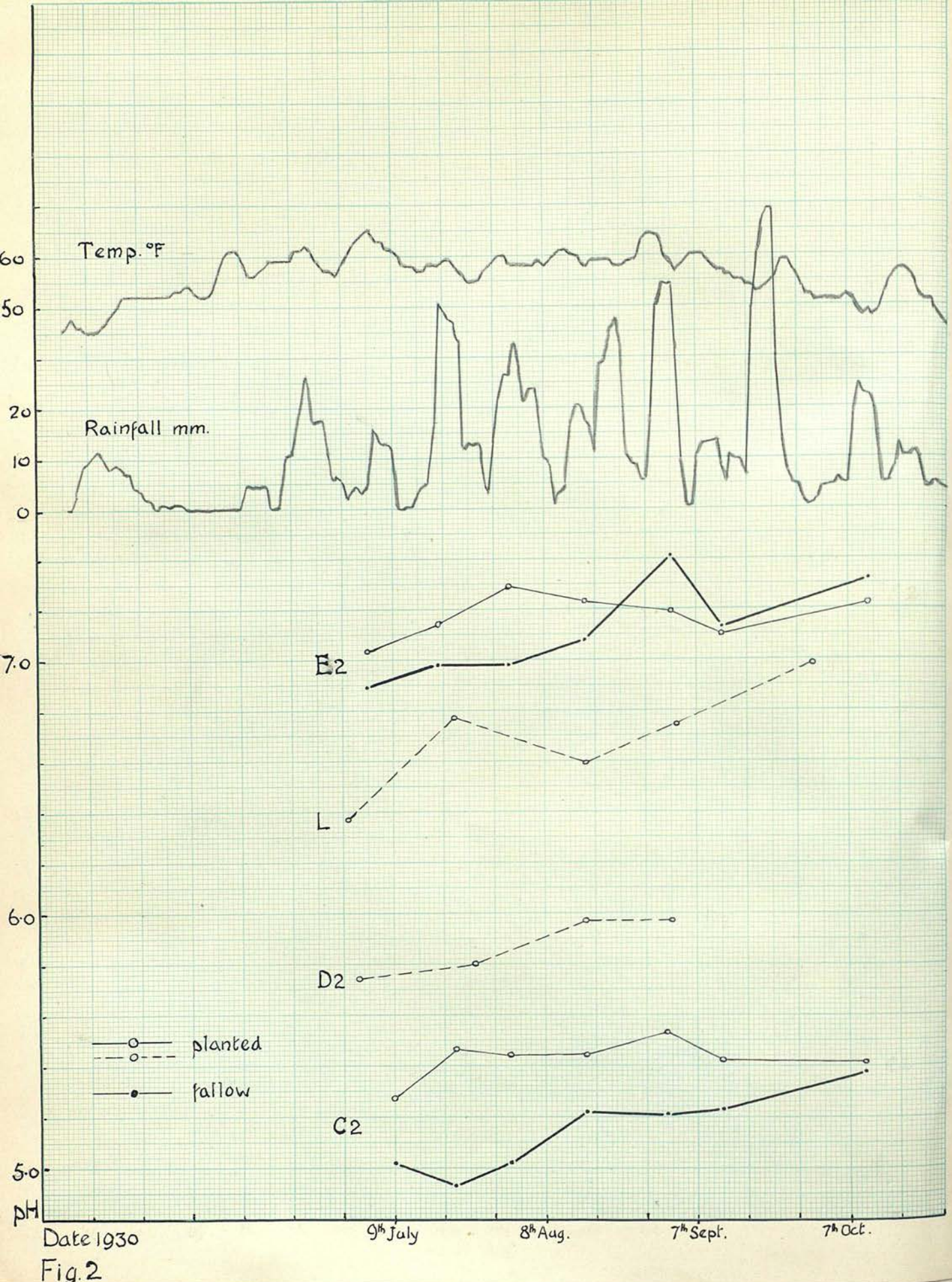
C1
1B2
Date 1929
29th June
29th July
28th Aug.
27th Sept.
17th Oct

Fig. 1.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5.0
pH
Date 1930
9th July
8th Aug.
7th Sept.
7th Oct.

Fig. 2

To reveal the possible effects of climate upon the changes in acidity, rainfall andmean temperature data for the two seasons have been shown in figs. 1 and 2. In order to smooth out the day to day fluctuations and, at the same time, make allowance for the lag between air and soil conditions, the average mean temperature and also the total rainfall for the preceding five days have been plotted. Since there are no data for fallow soils in 1929, a comparison of the results for the two seasons is limited to a few general features.

In 1929. (a) From 29th July to 18th August - moderate temperatures and high rainfall - there is an increase or little change in the pH values. (b) From 18th August to 20th September - high temperatures and moderate rainfall - there is a decrease in the pH values for the more acid soils and an increase in the pH values for the less acid soils. (c) The final readings, made at the beginning of October after a spell of cool, wet weather - generally show an increase in the pH values. The pH value of the heavily limed soil 1 B5 fluctuated slightly about the value 8.1 which is not far removed from the figure for soils in equilibrium with excess calcium carbonate (68).

In 1930. There are not enough observations to show the effect of the warm, dry period in June. (a) From 12th to 23rd July - normal temperature and high rainfall - there is an increase in the pH values with two exceptions, for both planted and fallow soils. The plants were not so far advanced as in the similar period (a) in 1929, which possibly accounts for the more definite change. (b) From 10th August to 2nd September - high temperature and high rainfall - the planted soils show somewhat similar tendencies in acidity changes as in (b) 1929, whereas all the fallow soils show a very marked increase in pH. In every case (see also fig. 3), except C2, the fallow soils become less acid

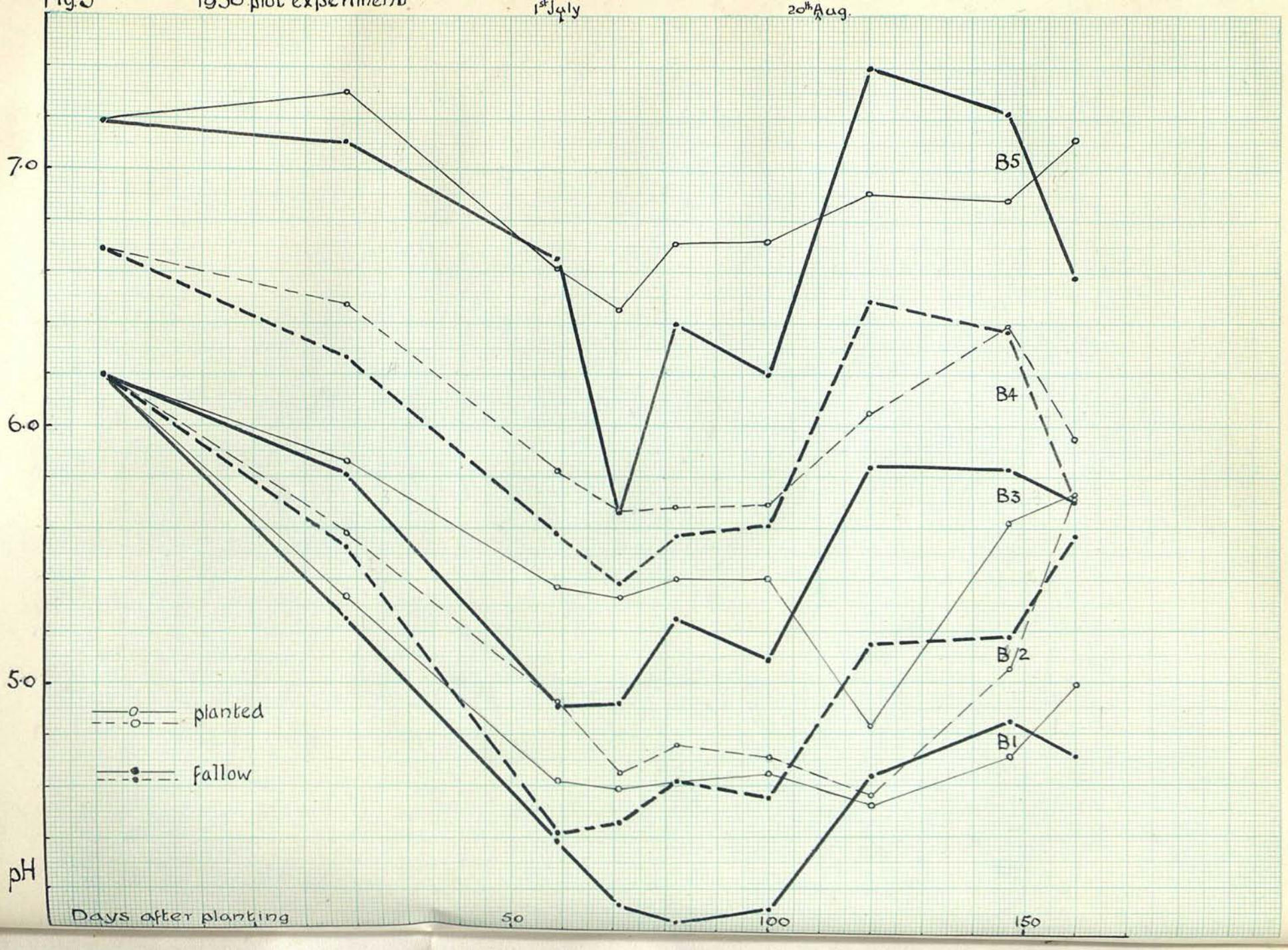
than/

than the planted soils in this period. The exception is possibly due to the fact that the ground under investigation at C2 was on a slope and much of the heavy rainfall at the end of August ran off. (c) The final readings, made in October after a period of cool, wet weather, show an increase in the pH values of planted and fallow soils, except in the cases of B4 and B5 (fig. 3), which were limed.

It will be observed that, although considerable changes in soil reaction may occur during the growing season, these changes are not by any means uniform in character or extent. For example, the maximum change in pH value for C1 is only 0.08 whereas that for L is 0.65 in the opposite direction. The observations agree with those of other workers (5, 27, 32, 60) regarding the amount and irregularity of the seasonal variations in reaction. None of the soils considered (except 1 B4 and 1 B5) had received lime for a number of years; the crop was the same in all cases and the manuring was similar; for each year the variation in climate, except in localities B and F of greater altitude, could be regarded as negligible over such a small area. Consequently, the irregularity of the acidity changes may be ascribed to soil characteristics. It is noteworthy, for example, that at L the average pH value of the samples increased from 6.2 to 6.9 in 1929 and from 6.4 to 7.0 in 1930.

(b) Plot experiments 1930-1934. The results of the acidity determinations from the date of application of the sulphur are shown in fig. 3. The first plants appeared at 25 days, the Epicure plants were flowering and small tubers had formed at 70 days, the crop was harvested at 147 days after planting. The Golden Wonder plants were, of course, later throughout and were not harvested until 160 days after planting. The differences in the/

Fig. 3 1938 plot experiment



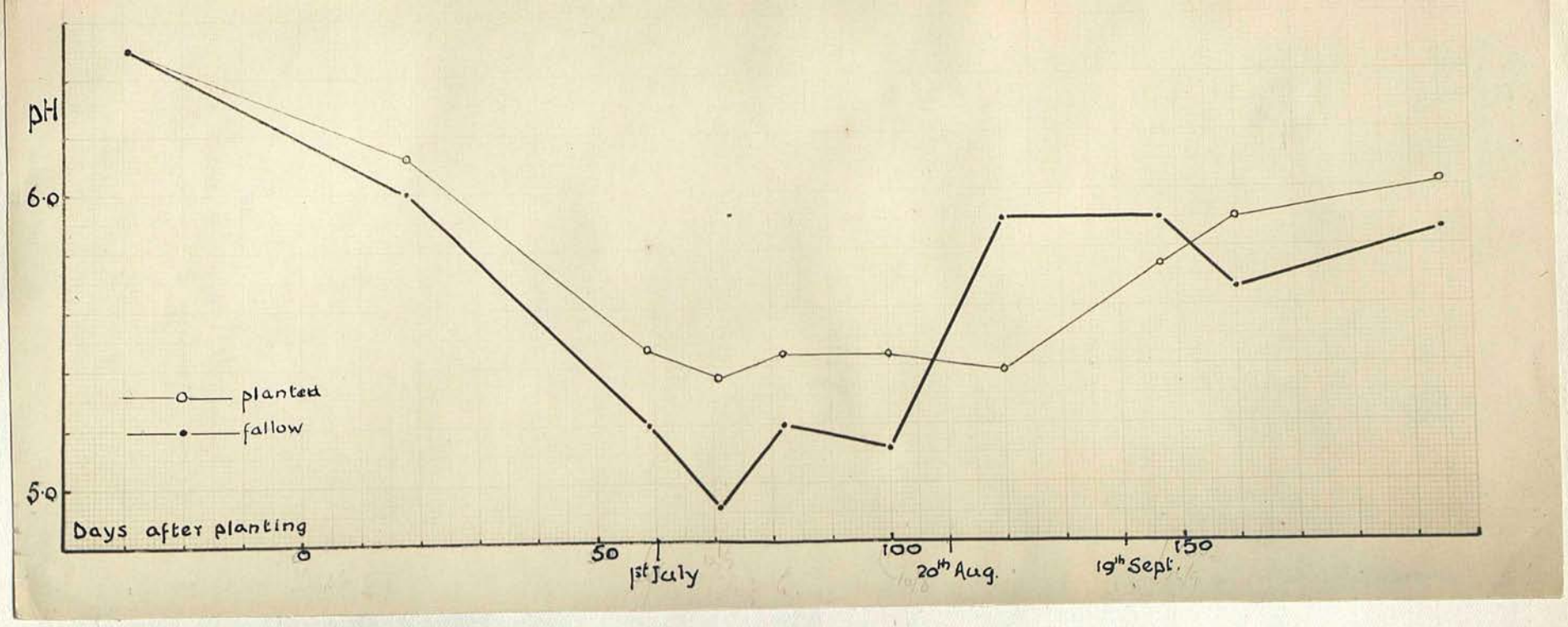


Fig. 4. 1930 plot experiment.

the acidity of the soil under the two varieties were, however, not greater than the experimental error due to sampling, and each of the "plant" curves, therefore, represents the average values of four samples.

The fluctuations and total changes in acidity are very great, but the remarkable parallelism amongst the five pairs of curves suggests the probability of a common factor in all five plots and some justification for simplifying the examination of the data by averaging the results for the five fallow soils and those for the five planted soils. The "average" curves are presented in fig. 4.

The curves gradually diverge until about 70 days after planting, at which point the pH value of the fallow soils has reached a minimum. The pH value of the planted soils remains practically constant during the next 50 days and then gradually increases. The value for the fallow soils, on the other hand, fluctuates considerably, but at 160-190 days has approached close to that for the planted soils. It is interesting to note how all the curves for plots 2, 3, 4 (fig. 3), tend to merge at 160 days.

The principal facts which emerge from these results are that the acidity of the soil increased during the growing season and then returned to a point not far removed from that at the time of planting, and that the changes were considerably reduced by the presence of the growing plant which exerted its greatest influence at or about the stage of maximum growth.

Generally speaking, these results confirm the less complete data from the field experiments. It seems that in the early part of the season the acidity of the soil increases irrespective of climatic factors, which may be due to the accumulation of salts as a result of the general rise in temperature and/

and increase in biological activity and chemical reaction in the soil. This accumulation may not be so great where plants are growing so that the increase in acidity is not so marked. During the period of greatest growth in July and August, rainfall appears to be more important than temperature in effecting changes, for in both seasons heavy rainfall produced decreases in soil acidity, and in 1930 the changes in the fallow soils were very large. There is a general tendency for the acidity to decrease towards the end of the growing season and, where observations are sufficient, it is evident that the pH values for planted and fallow soils are becoming similar.

Numerous investigations have been made on the concentration of salts in the soil. In a recent paper, Stremme (63) has discussed the variability of the plant nutrients with respect to climatic factors and soil weathering. He points out that, although there may be a fairly close relationship between the movement of water soluble constituents and meteorological factors in spring, the position is extremely complex at other times of the year and the changes are not confined to the surface soil. Results obtained by Bouyoucos with the freezing point method (8) show how the salt content may increase, during the summer months, to a large extent in a fallow soil and to a slight extent in a soil under crop, and how the changes may be considerably influenced by rainfall. Studies of the aqueous extract of the soil by Stewart (62) and of the displaced soil solution by Burd and Martin (9, 9a) have also revealed the effect of the plant in reducing the concentration of all the common soluble nutrients with the exception of bicarbonate. The tendency for the salt concentration to become similar at the end of the season under fallow and crop conditions has also been demonstrated by similar work along these lines (24).

Such/

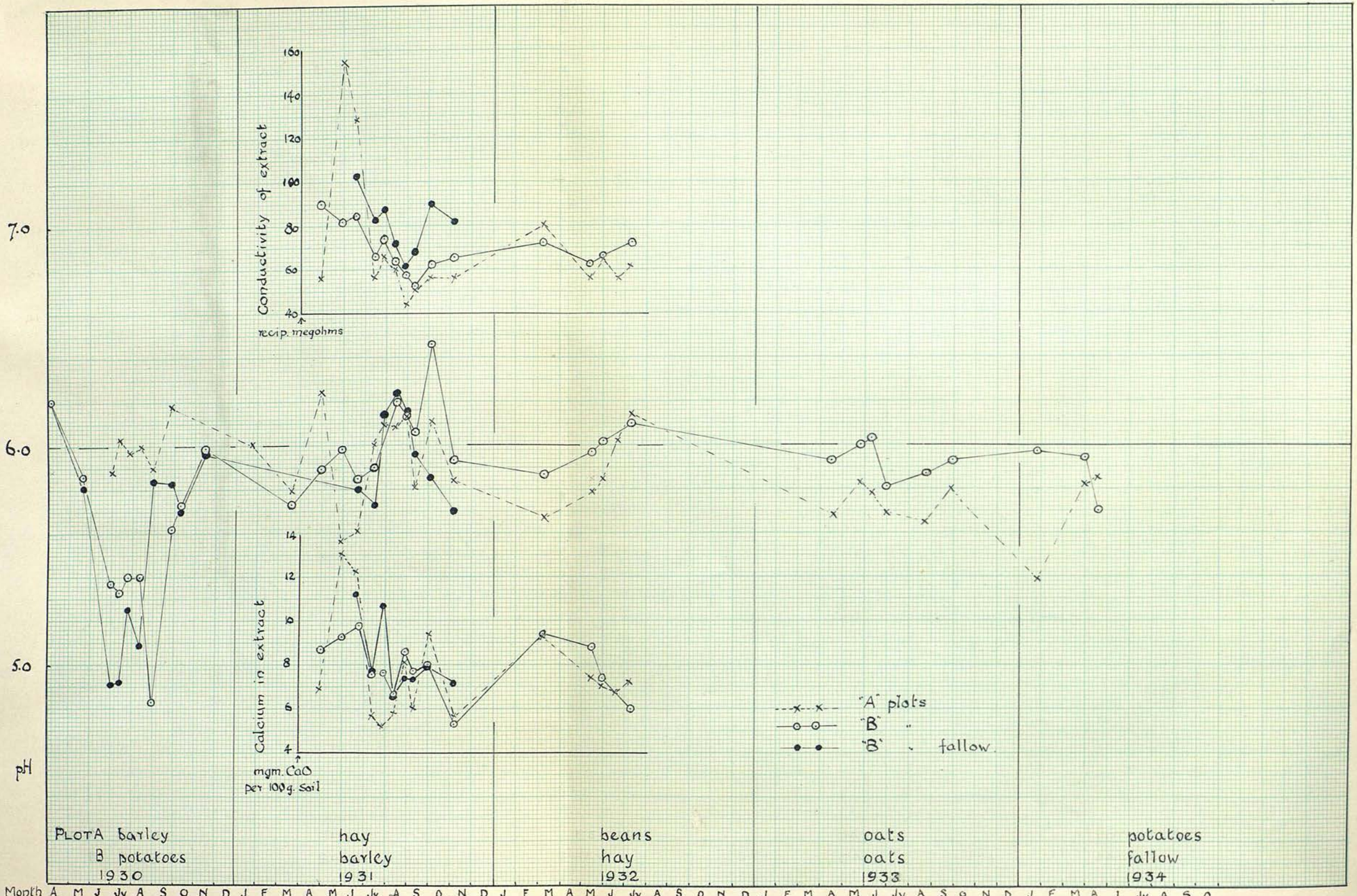


Fig. 5. PLOT EXPERIMENT. SOIL B, untreated plots.

Such evidence would make it appear that fluctuations in soil acidity are definitely connected with changes in the concentration of soluble salts and that the difference in acidity between cropped and uncropped soil is due, at least in part, to the absorption of salts by the plant. The plant may, of course, affect the concentration of salts indirectly, and it is also probable that the bicarbonate ion, formed as a result of plant growth, exerts some influence.

In order to make a preliminary examination of these possibilities, representative samples of soil (about 10 borings) were taken from each plot of sets A and B at frequent intervals during 1931 and 1932. The acidity was determined as usual, but, in addition, determinations of electrical conductivity and calcium content were made on 1 : 5 extracts of the soil and distilled water. The former measurement was obtained by means of a Digby and Biggs Dionic Water Tester, checked with different standard solutions against a conductivity cell. The calcium was determined as described on p. 65 . In each set, the fluctuations in these values over the period of observation were very similar for the different plots and only the results for the two control plots A3 and B3 are given in fig. 5.

That the fertility of the soil and the effects of cropping are reflected fairly accurately by the nature of an aqueous extract of the soil have been established in numerous researches (9a). It is recognised that the results for conductivity and calcium are not strictly comparable for the cropped and uncropped areas owing to the mutual effects of ions in solution; but, without stressing their absolute values, the results may be regarded as sufficient to permit of a general comparison.

The chief features of the results are (1) the marked fluctuations in soil acidity during the growing season, (2) the effect/

effect of the crop in modifying these fluctuations, (3) the close parallelism between the conductivity and calcium content of the soil extract and (4) the inverse relationship between conductivity and pH value. The first two points substantiate the results previously obtained with potatoes in 1930 on plots B, although the effect of the barley has been less than that of potatoes; the third observation is what one would expect since calcium normally forms a considerable proportion of the total electrolytes in drainage water: the fourth is fairly conclusive evidence of a general relationship between the concentration of electrolytes in the soil at any particular time and the pH value of the soil suspension in water. There are a number of discrepancies, however, which are difficult to explain. For example, in the autumn of 1931, the inverse relationship between pH and concentration of electrolytes breaks down in one or two cases and the distribution of rainfall in that period was so uneven as to make comparisons useless. As a general rule, it has been found extremely difficult to correlate climatic conditions with change in acidity. The very large fluctuations obtained in 1930 are possibly the result of exceptional conditions as regards distribution of temperature and rainfall. The temperature and rainfall distribution has continued to be rather abnormal right up to the present year, but with different effects. In 1932 and 1933 the fluctuations in acidity are definitely greater than experimental error but not nearly so marked as in the previous years. It is possible that during a dry summer or one which is characterised by a fairly regular distribution of rainfall and temperature, rather than by periodic thundery conditions such as occurred in 1930 and 1931, the surface soil becomes dry and its properties remain more or less constant. Usually, field drains flow in the summer time only after a very heavy precipitation on an already moist/

moist soil. It may be said, however, that throughout the period of observation there is a tendency for acidity to increase in the spring, fluctuate during the summer and return to the original value during the winter. The other data collected from these plot experiments, however, merely illustrate that the acidity changes are related in some way to the concentration of soluble salts in the soil.

One of the recommendations made by the International Committee on soil reaction measurements (60) is that determinations should be made on suspensions of the soil in N.KCl as well as in water in order to "measure a more permanent characteristic of the soil". A considerable number of the samples concerned in this investigation were so examined and the results were quite similar to those obtained for acid soils by Pozdena (43). The pH value for the KCl suspension was always lower, about one unit on the average, than that for the water suspension, and the seasonal fluctuations were not eliminated, Recent publications indicate, moreover, that, although the relationship between acidity and concentration of the soil solution may be fairly simply expressed (69), the factors which govern the effects of neutral salts on soil acidity are still obscure (29). The value of making measurements on KCl suspensions at present is therefore questionable, and since it is desirable to study the soil in as natural a state as possible, a preliminary washing of the soil sample would seem to be preferable. As might be expected, the pH value of the soils under investigation are considerably increased by such treatment to figures approximating to those obtained during winter; the washing was usually carried on until the conductivity of the filtrate became relatively low. An indication of the effects of washing is given below.

(c) The effects of fertilisers. From the results obtained in the/

the above plot experiments with soil B, it is obvious that such variation in acidity may be produced without the possible influence of artificial fertilisers. Fairly large dressings of "potato manures" were added to the soils under observation in the 1929 and 1930 field experiments, however, and it is desirable to take the question into consideration.

Although the influence of dressings of ordinary fertilisers on soil reaction would seem to be a matter of considerable importance, comparatively few useful or reliable experimental data are available except on the effects of ammonium sulphate. Many observations have been made in field experiments but, apart from the fact that mixed fertilisers are commonly involved, this method is liable to suffer from at least one serious disadvantage. The quantities of material usually employed are obviously so small as to make any changes difficult to establish in a reasonable time on account of the possible seasonal fluctuations in soil acidity described above. Changes in acidity almost certainly involve ionic exchange and, in view of the importance of calcium in this respect, a series of experiments was carried out with the more common fertilisers which contain that element.

The method employed in some preliminary work was to prepare a titration curve for fertiliser and soil by shaking a 1 : 2.5 suspension of soil in water with different quantities of the fertiliser overnight, and measuring the final pH value of the suspension. A series of curves could then be drawn showing the effects of different substances on the same soil. In order to approach more nearly to equilibrium conditions and avoid secondary effects due to cation exchange, the later experiments were carried out over a larger period and the soil was filtered and washed before determining its pH value. The soils which were employed in/

in the experiments were from the same areas as B, W and P previously described, and details of the investigation (30) are given in appendix IV. It is shown that superphosphate increases the acidity and that all the other fertilisers decrease the acidity but that the changes which would be effected by normal dressings are minute except in the case of basic slag. The effect produced by slag depends upon the soil type and the nature of the slag, but is never so great as that produced by calcium carbonate. It is also shown that washing the soil before making the pH determination is responsible for considerable increases in the pH values, which usually become slightly higher when the soil has previously been treated with a fertiliser.

Such results fall into line with the generally accepted theories on base exchange, and provide additional evidence that fluctuations in soil acidity are caused, at least partly, by the change in the concentration of soluble salts during the season. These salts are able to bring about a type of exchange acidity, as discussed by Robinson (47), when the soil is unsaturated and is shaken up with water for a pH measurement. There is no doubt, therefore, that a determination of the acidity or certain other properties of a sample of soil taken for routine examination during spring or summer may be very misleading.

These results have been confirmed in two series of pot experiments, one with soil B and the other with soil W.

(d) Pot experiments. (i) Soil B, 1930-1934. A large sample of soil B, taken from the field in March 1930, was employed in these experiments. It was broken up while still moist, passed through a $\frac{1}{4}$" riddle and divided into three portions. One portion (B2) received 0.024 per cent. sulphur, the second (B3) was untreated, the third (B4) received 0.12 per cent. calcium hydroxide. The additions were calculated in terms of oven dry soil. Ten pots (12" diameter)/

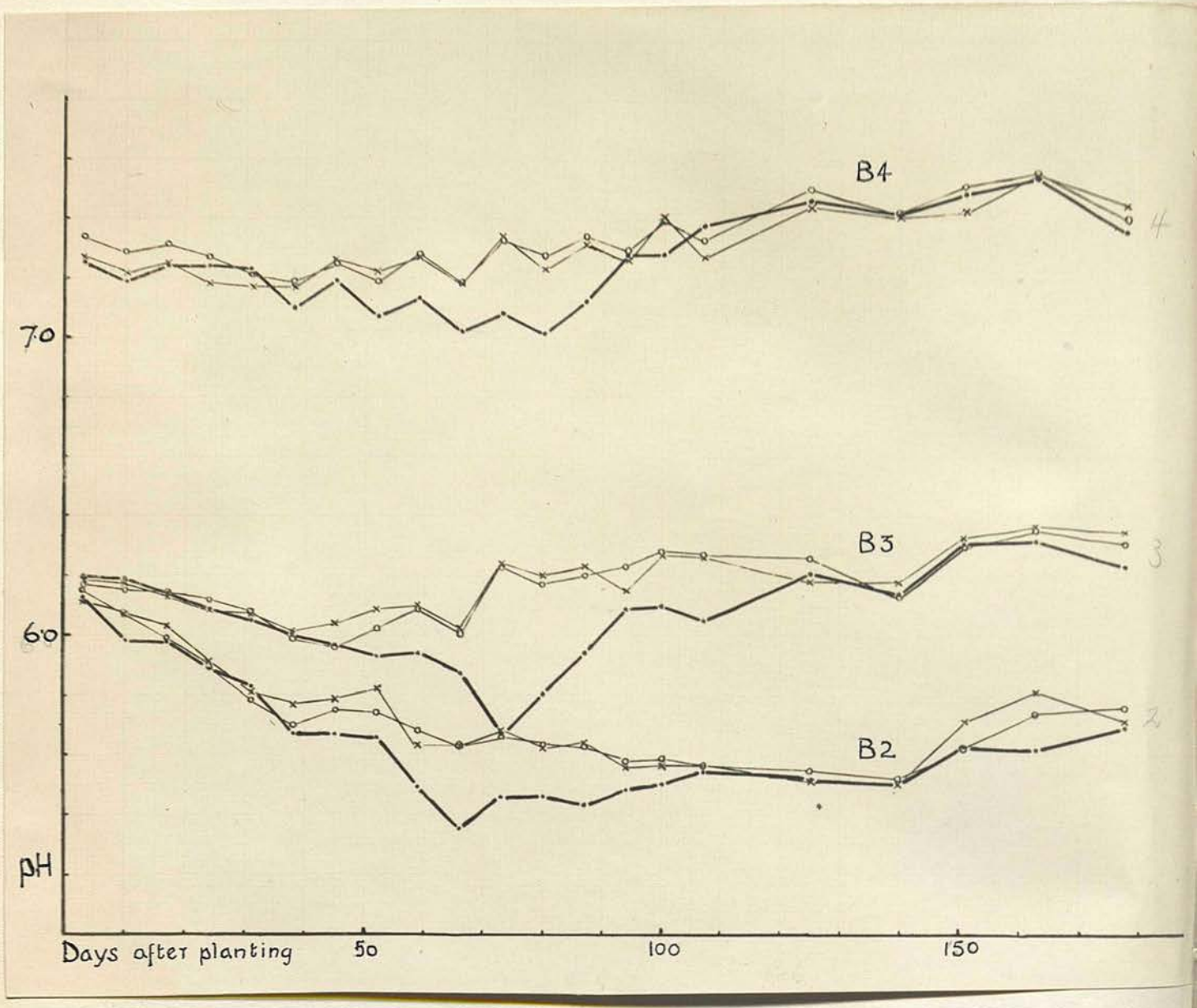


Fig.6. pot experiment, SOIL B. 1930

—o— Golden Wonder
—x— Epicure
—•— Unplanted

(12" diameter) were filled from each portion on the 12th April and potatoes were planted two days later. Four pots of B2 were planted with the variety Epicure, four with Golden Wonder and two pots were kept fallow. The same was done for the series B3 and B4. Samples of soil were taken at weekly intervals until August, and then less frequently until October, by means of a small auger which reached to the bottom of the pots. During the warm, dry weather of May and June the pots had to be watered frequently, and that probably accounts for the fluctuations and total changes in acidity being small compared with those found in the field experiments already described, or in the incubation experiments (pp. 38-54). The results are shown graphically in fig. 6.

Considering the fallow pots first, it will be observed that the pH value of each soil decreased regularly to a minimum which, except for B4, existed only for a brief period. Those minima were reached after practically the same length of time as in the incubation experiments. The pH value then increased, rapidly at first and then more slowly, so that by the 9th October it had returned to the original value except in the case of B2. During the first 30 to 40 days the soils of the planted pots did not differ materially as regards acidity from those of the fallow pots. The Epicure plants appeared above the surface of the soil in the period 30-40 days, and the Golden Wonder in the period 40-50 days after planting. (The curves for the late variety, Golden Wonder, will be observed to lag somewhat behind those for the early maturing variety, Epicure.) From the time when the shoots appeared until about 60 or 70 days later when the haulms were withering, the pH values of the planted soils were definitely higher than those of the corresponding fallow soils. In all three series there may be observed a slight increase in pH value when the/

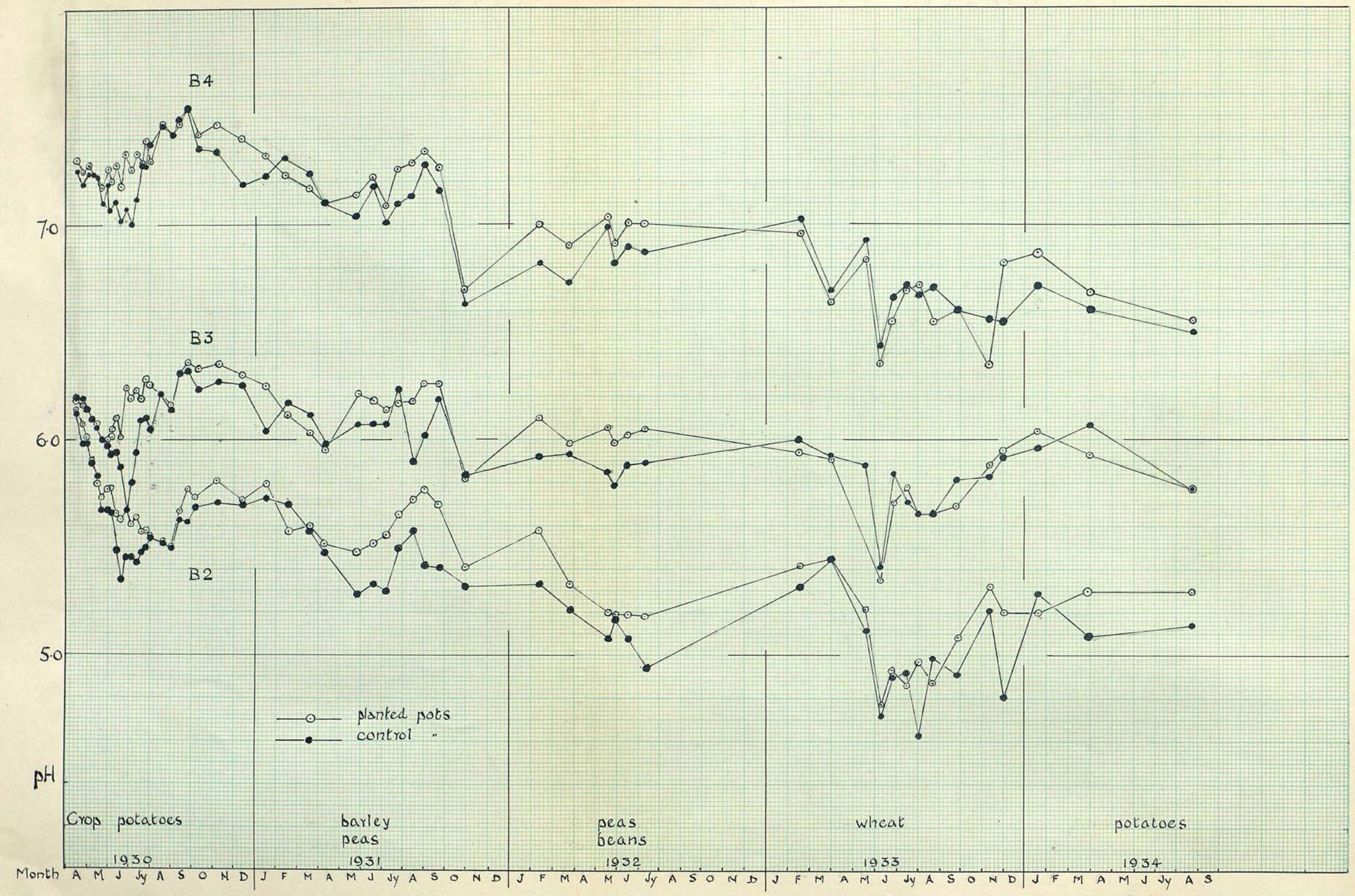


Fig. 6a. POT EXPERIMENT. SOIL B.

the shoots appeared, which is interesting in view of the similar observation in the case of the incubation experiments. The difference in acidity between planted and fallow soils increased until the plants were flowering, which corresponded approximately with the period of minimum pH values for the fallow soils: the difference then became less until eventually the curves for planted and fallow soils were practically the same.

The tubers were harvested during the period 145-165 days. The average yield per pot was 166 g., and the average tuber weight was 19 g.

This study of the seasonal fluctuations in soil acidity has been continued up to 1934, one series of ten pots B2 having been treated each spring (except 1934) with 0.015 per cent. sulphur. The crops have been barley and peas in 1931, peas and beans in 1932, wheat in 1933 and potatoes in 1934. No fertiliser has been applied apart from a light dressing of ammonium nitrate in June 1934, but the soil has been turned out of the pots and riddled each year.

The results, given in fig. 6a, are the averages for each pair of fallow pots and each set of 8 pots under crop. They are similar to those obtained in 1930 with potatoes, viz. a general fall in pH value during the growing season followed by a rise in autumn and a maximum difference between planted and unplanted pots during the period of maximum growth, i.e. between 70 and 100 days after sowing. The four annual applications of sulphur to the first series have produced a progressive increase in acidity in addition to the seasonal fluctuations: there has also been a steady decrease in pH value of the limed soil, but the untreated soil has not shown any marked tendency in this respect. Those soils which have been fallow throughout are apparently becoming more acid than those which have been planted, but/

but it is probably too soon to discuss the question of permanent changes.

(ii) Soil W, 1931-1934. Four series, A, B, C and D, of ten Mitscherlich pots were filled with soil W in April 1931, the series being treated respectively with 0.0, 0.20, 0.45 and 1.0 per cent. of calcium hydroxide. The original soil is a very unsaturated and infertile sandy loam and those treatments were calculated, from a titration curve, to raise the original pH value of 4.6 by 1, 2 and 3 units respectively. This method of measuring the buffer capacity of a soil has been described by Smith and Coull (58) and its application in the estimation of "lime-requirement" by Smith (55). Details of the procedure are given in appendix V, pp. 84-87 , where soils D, F and H are particular samples of B, W and P respectively. Each series consisted of two fallow pots, and eight planted pots: the soil in each pot was sampled by means of a small auger at regular intervals for pH determinations, and the average value are shown in fig. 7. At the end of March 1933, half of the cropped pots in series A and B were treated with 0.2 per cent. of basic slag before sowing.

The results demonstrate what has been observed in all other cases, viz. fluctuations in acidity associated with moisture and temperature variations and a decrease in acidity due to the presence of the growing plant. The effects are not so apparent in series A on account of the very poor growth, or in series D where the soil and heavy dressing of calcium hydroxide have probably not reached an equilibrium. Even the smallest addition of calcium hydroxide was responsible for a large increase in crop yield and the larger dressings produced crops which were 6 to 16 times greater than those from the untreated soil. The effect of basic slag, discussed in appendix IV, is also demonstrated.

The/

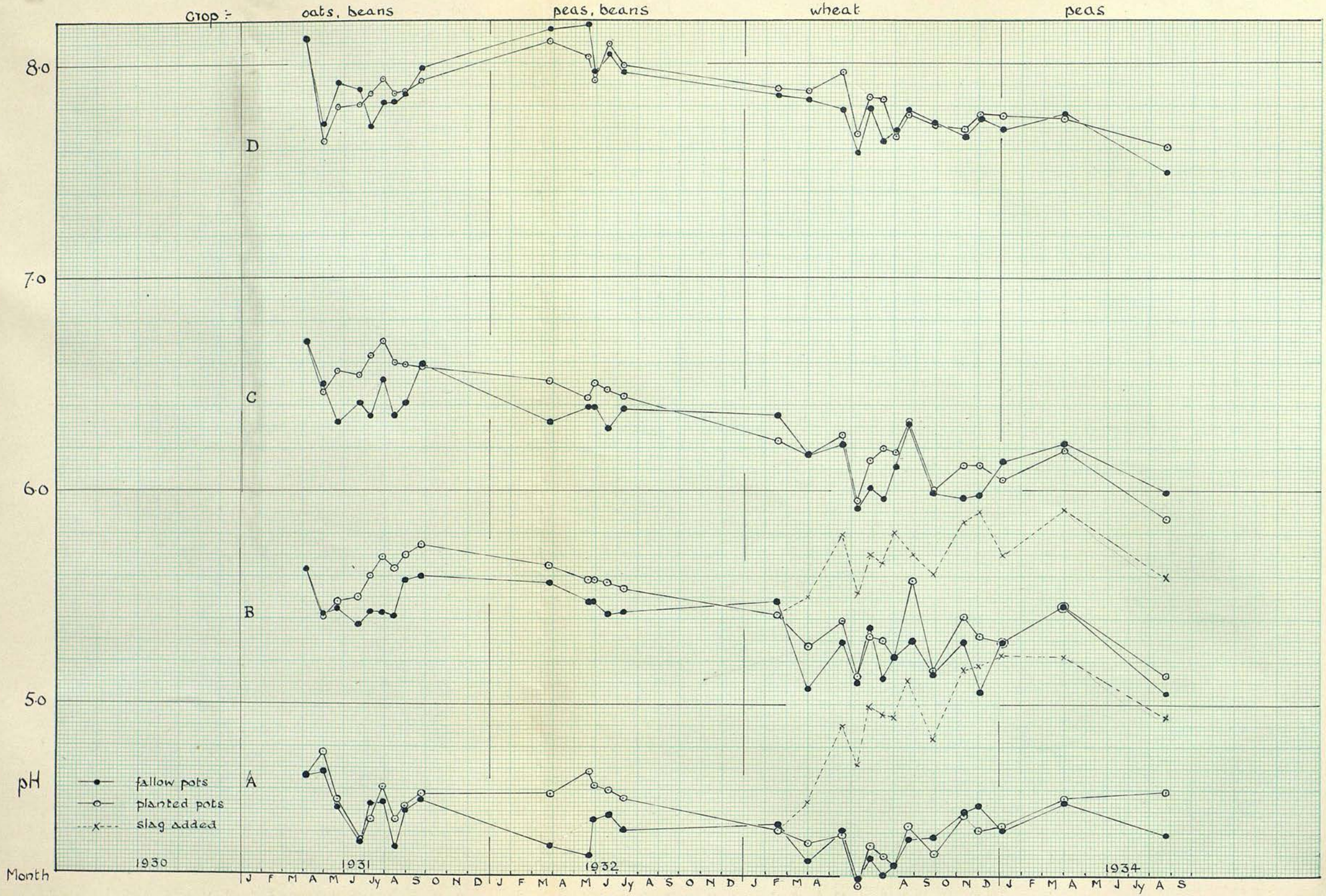


Fig. 7. POT EXPERIMENT. SOIL W.

The drainage water was not returned to the pots, but it was sampled six times during the growing season 1931 and its content of calcium was determined. (It is worthy of note that the amount of organic matter in the drainage water increased under the influence of successive additions of calcium hydroxide to the soil.) There were large variations in the quantities of drainage water from the different pots, so that undue stress could not be placed upon the figures for calcium concentration. The average results, however, undoubtedly reflected the concentration of the solution in equilibrium with the soil particles during the growing season. They indicated how the concentration of solutes may be reduced by the presence of plants, how rapidly the calcium is lost from the soil and how the magnitude of the loss is increased by liming. These points have, of course, been demonstrated in numerous lysimeter studies (34). The fluctuations for individual pots were rather large, but the following average figures, obtained between April and September, show the relationship between acidity and drainage for the fallow (a) and cropped soil (b) in each series.

	A		B		C		D	
Series	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Average pH	4.49	4.53	5.44	5.57	6.41	6.58	7.82	7.82
Average concn.[1]	30	25	108	85	158	143	381	380

[1] CaO in mgm. per litre per pot.

(e) Incubation experiments. (i) With potatoes. Soils J, P, W, G and L were passed through a 2 mm. sieve and used for these experiments. In addition, samples of B, which had an initial pH value of about 6.2, were treated with calcium hydroxide and with sulphur to obtain a range of pH values from about 8 to 4. Similarly, samples of P, having an initial pH value of 3.6, were treated with different amounts of calcium hydroxide to obtain pH values/

values of about 5 and 6. Soils W and G, of which the original pH values were about 4.5 and 6.7 respectively, were examined only in the untreated condition. A summary of the soils examined is given in the accompanying table, the treatment being calculated in each case for oven dried soil.

Soils examined in incubation experiments.

Soil	Sample	Treatment
B	1	0.12% S
	2	0.024% S
	3	none
	4	0.12% $Ca(OH)_2$
	5	0.35% $Ca(OH)_2$
P	1	none
	2	1.0% $Ca(OH)_2$
	3	2.0% $Ca(OH)_2$
W,G,L	-	none

The soils, which had not reached an air dry condition, were brought to moisture contents suitable for plant growth, and 500 g. portions were placed in glass dishes. Small pieces of potato tubers of the varieties Epicure and Golden Wonder were then planted so that for each sample, excepting B1 and B5 which were not planted, there were six dishes, viz. two control, two planted with Epicure and two with Golden Wonder. In the experiment with soil B, the dishes were placed in a ventilated glass frame, in which the temperature was maintained between 18° and 20°C. The experiment with the other three soils was carried out in mid-summer and the dishes were left at laboratory temperature which fluctuated slightly about 18°C. The dishes were watered daily and small samples of soil were removed at regular intervals for pH determinations. The values obtained are shown graphically in figs. 8 and 9. Each curve represents the average of duplicates until certain of the plants died. For example, in fig. 8 the curve for Epicure in sample B3 represents the average value for the two plants up to 35 days; about that time the plant in one dish/

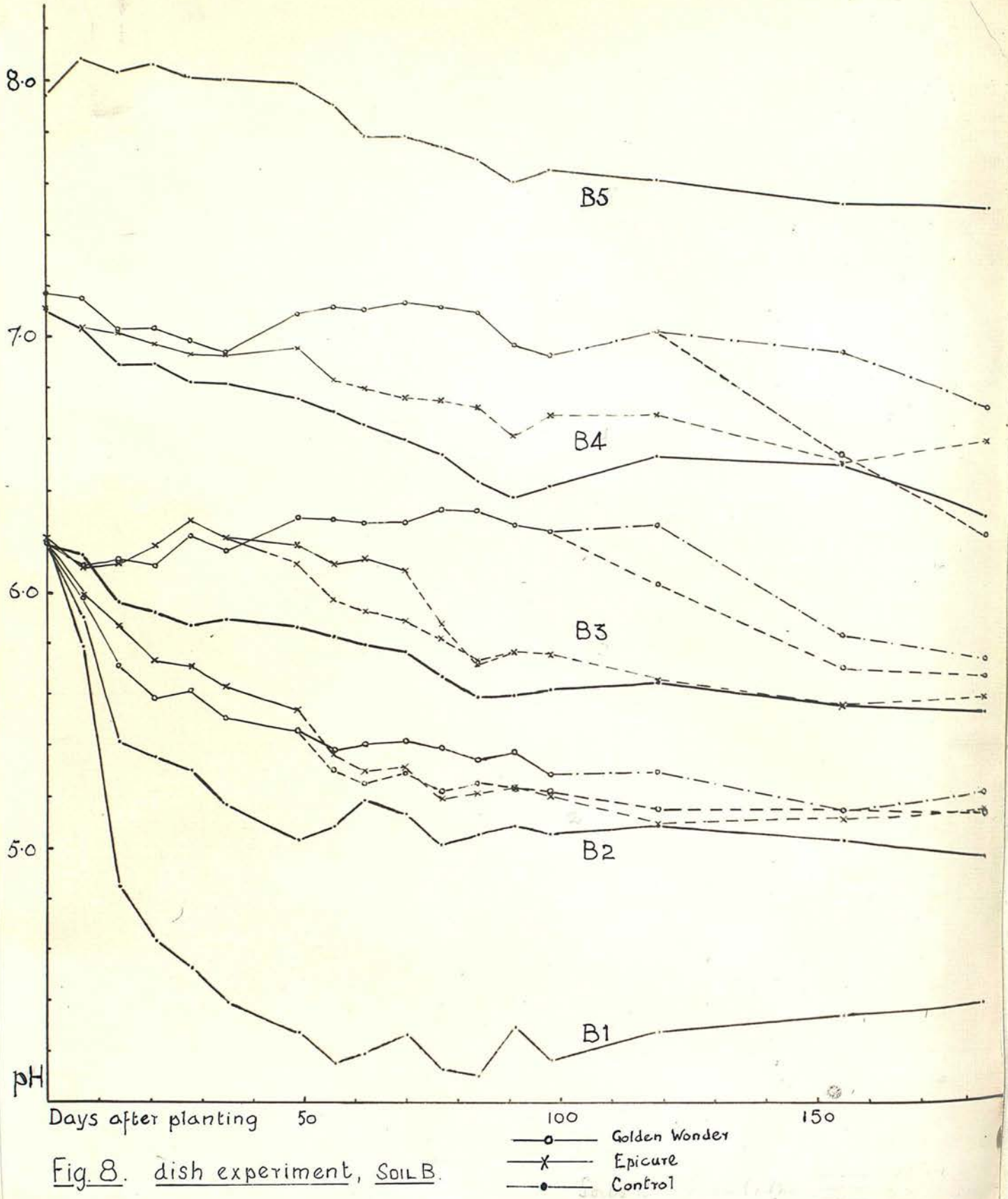


Fig. 8. dish experiment, SOIL B.

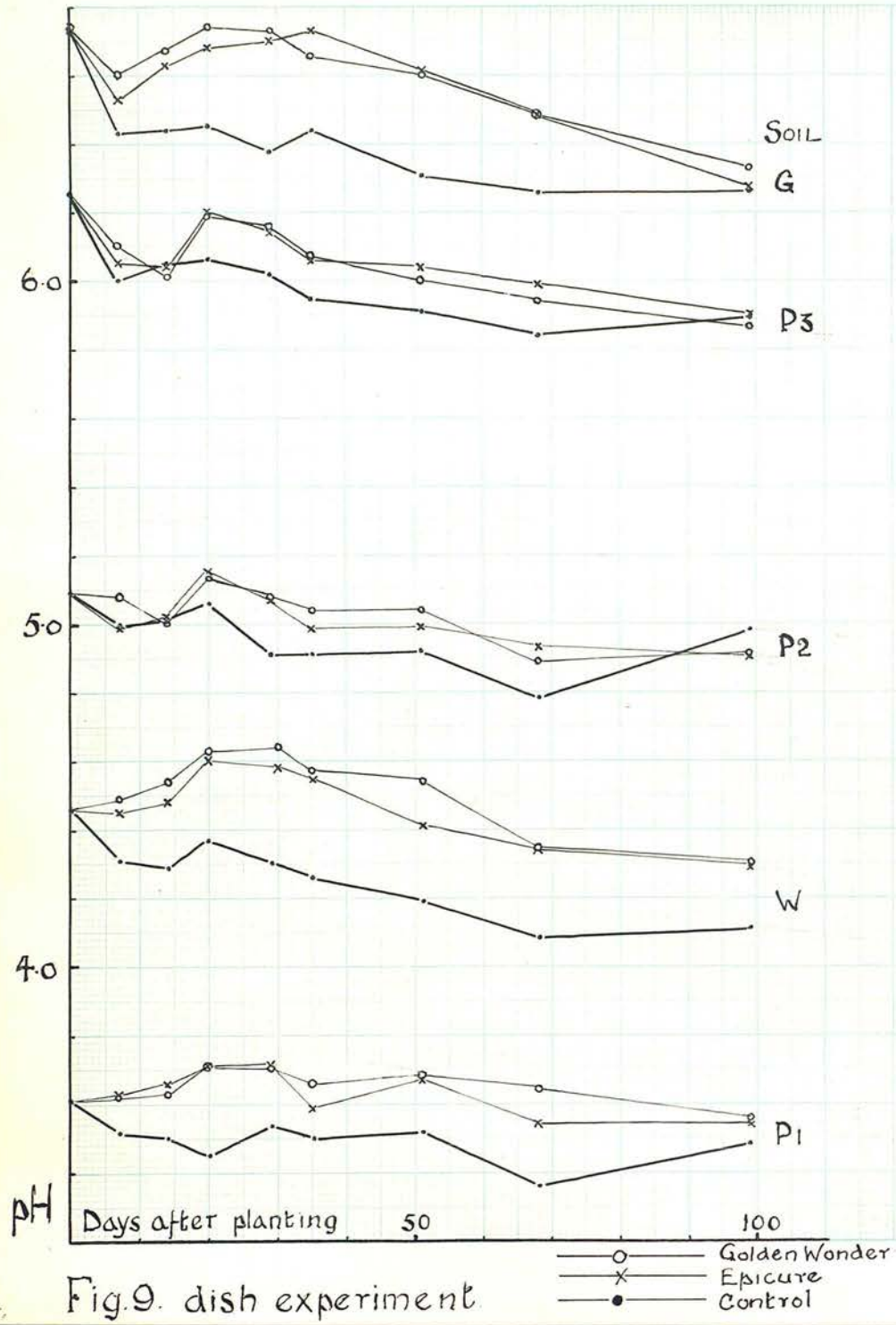


Fig. 9. dish experiment

dish died, and the broken line shows the subsequent value for the soil in that dish: at 70 days the plant in the second dish died and there was a rapid decrease in pH value until at 90 days the soils in the two dishes were again giving practically the same pH values. Where the solid lines give place to alternate dash and dot, as, for example, with most of the Golden Wonder plants at 90 days, it indicates that the plants were "nipped" to stop growth. Those plants were very vigorous, however, and in some cases still survived when the experiment was concluded, although growth had practically stopped.

The following points may be observed in a study of the curves. In the case of all the unplanted soils there was a fairly steady decrease in pH value during the first 70-100 days and thereafter the changes are small and irregular. The changes with the highly buffered peat soil are, naturally, less pronounced. In the case of soils B1 and B2 the results were very similar to those of the action of sulphur on soils reported by other workers (2, 10. 26), there being an extremely rapid increase in acidity during the first few weeks. In the soils supporting plants, there was usually a slight fall in pH value until the shoots were established, and then a rise during the rapid stages of growth (except in the case of B2) to a point higher than the initial value. The pH then decreased, fairly rapidly after the death of a plant, until it approached the value for the unplanted soil. That B2 should prove an exception was apparently due to the fact that the normal influence of the growing plant, in preventing an increase in acidity, was more than counterbalanced by the effect of the oxidation of the sulphur in increasing the acidity. Evidence in support of this explanation was supplied first of all by the fact that the sulphur produced a greater increase in acidity in the fallow than in the planted soil, and secondly/

secondly by the results recorded in fig. 10. In this experiment, small tuber sprouts were planted in a quantity of soil which had been treated with an amount of dilute sulphuric acid equivalent to an addition of 0.02 per cent. sulphur.

The curves are quite similar to those for B2 after 70-80 days when the oxidation of sulphur was apparently complete. The decrease in acidity due to the rapidly growing plant and the subsequent return, on "nipping", to that of the unplanted soil are very well marked in fig. 10.

In order to make sure that the differences in pH values between unplanted and planted soils were not due to differences in moisture content affected by the growing plant, a number of soils were maintained at definite moisture contents by daily watering for a period of 30 days. Measurements of pH were made at intervals and the final values are reported below.

pH values after 30 days at constant moisture content.

Soil	Per cent. moisture			
	10	15	20	25
B + 0.025% S (as H_2SO_4)	-	5.23	5.25	5.28
B	-	5.89	5.85	5.84
B + 0.125% $Ca(OH)_2$	-	6.86	6.87	6.90
W	4.42	4.37	4.38	-
G	6.24	6.28	6.31	-

The results confirmed those shown in figs. 8, 9, 10 and showed that different amounts of moisture in the soil, over a range of 10 per cent. in the neighbourhood of the optimum moisture content, had very little effect upon the changes in acidity.

It has already been shown (p. 14) that a large increase in the quantity of electrolytes in a soil may occur if it is maintained under conditions suitable for biological activity. Such an increase modified by the absorption of salts by/

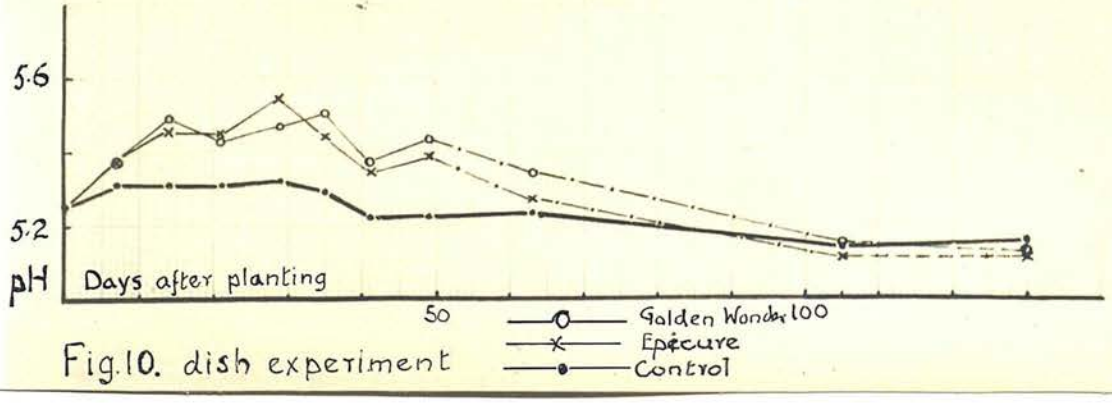


Fig.10. dish experiment

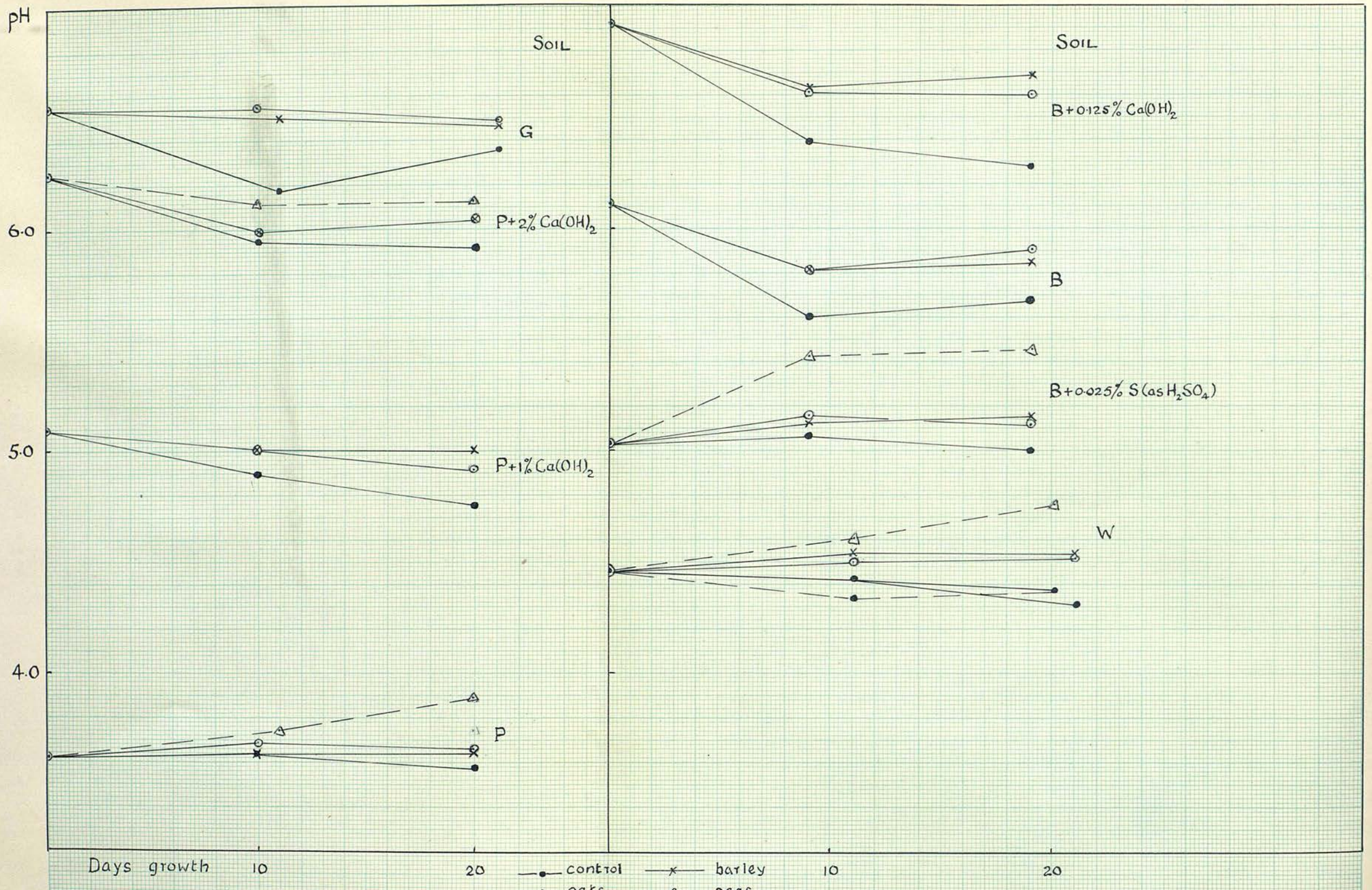

pH
6·0
5·0
4·0
Soil
G
P+2% Ca(OH)2
P+1% Ca(OH)2
P
Soil
B+0·125% Ca(OH)2
B
B+0·025% S (as H2SO4)
W
Days growth
10
20
10
20
control
barley
oats
peas

Fig. 11.

by the plant would account for the results obtained for the fallow soils and the early observations for the planted soils. A return of electrolytes from the decaying plant to the soil is a possible explanation, suggested by the observations of Burd and Martin (9a), of the later results for the planted soil.

(ii) With other plants. Barley, oats and peas have also been employed in the laboratory experiments, but their rates of growth are so much greater than that for the potato that a simpler method of studying the changes was possible. A large sample of soil was brought to a suitable moisture content and small quantities - 15 to 25 g. according to the nature of the soil - were placed in crystallising dishes or beakers. The same number of seeds - usually about 3 - which had been allowed to germinate, were then planted in each dish, with the exception of the controls, and the dishes were placed in a large glass frame maintained at 18-20°C. The dishes were watered regularly and, at intervals of a few days, a complete set in duplicate was taken for examination (see photograph 3). The plants were removed and the soil adhering to the roots was washed into the dish with CO_2-free water. The pH value of the whole sample of soil was then determined.

Some of the results obtained by the above method have been brought together in fig. 11. They are quite typical of a large number of similar experiments, in which it was invariably found that the acidity of the soil was decreased by a growing plant. The pH value of the moist soil might be increased or decreased by incubation for 10 or 20 days, but the value was always greater when the soil was supporting an actively developing plant.

These observations on the action of barley, oats and peas/

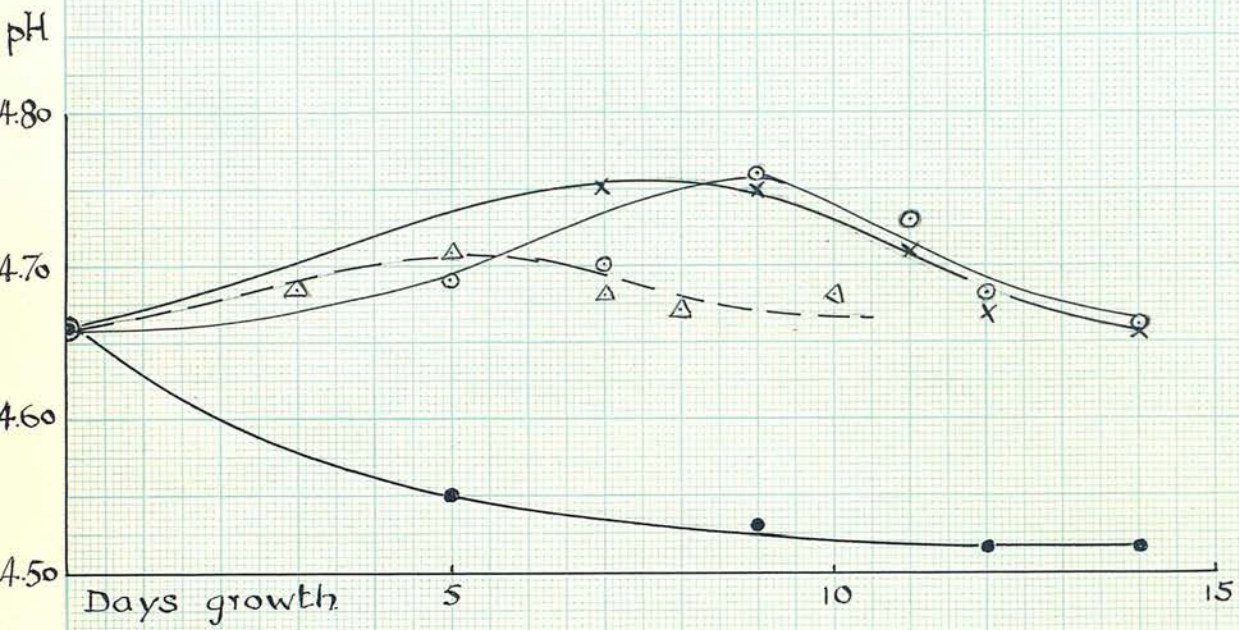

pH
4.80
4.70
4.60
4.50
Days growth
5
10
15
control
barley
oats
peas
Soil W

Fig 12.

peas during their early stages of growth are substantially the same as those reported above for potatoes; and the only differences which have been found in numerous trials have been of degree. For example, the magnitude of the effect has depended to a certain extent upon the character of the soil. The presence of calcium hydroxide or calcium carbonate in the soil or the addition of sulphur or sulphuric acid to increase its acidity, affects the results only slightly. With respect to the relative influence of different plants, fig. 11 shows that peas exerted a greater effect than oats or barley. This may be due to the fact that, according to Newton (39), more carbon dioxide is given off from the roots of pea plants than from the roots of barley plants of the same size. The relative effects have not always been the same in these experiments, however, and have seemed to depend largely upon the rapidity of growth of the particular plants under examination. In fig. 12, for example, the results of a set of experiments started on the same day are given. For some reason, the peas did not grow nearly so vigorously as the other plants, and produced a smaller effect on this occasion. The rate of growth and the general vitality of the plant also seem to be more important than the actual stage of growth and size of the plant or extent of its roots. No great differences were observed, for example, in the effects produced by pea seedlings which had been allowed to reach different stages of development in sand culture before being placed in the soil.

There is no question, however, that the effect is produced mainly in the earlier stages of plant growth. Slight discrepancies sometimes occur when experiments are repeated, but these seem to to be due to fluctuations in the acidity of the soil/

soil, which may occur during the first 2 or 3 days incubation. Robertson (45) has shown that a decrease in the pH value of the fallow soil is frequently preceded by a slight rise. Hence, the initial and final pH values may be determined to some extent by the length of time the soil has been lying moist before the experiment is started. Minor variations in the final pH values do not, however, alter the general conclusion that the plant has an influence upon soil acidity which is not confined to any particular case or set of conditions.

It is probable, from what has already been said, that the concentration of salts is rather smaller in the planted soil than in the unplanted soil, but it is difficult to imagine that the absorption of salts by the plant is the sole reason for the effects observed. It has been shown (18) that biological activity, as estimated by the production of carbon dioxide and nitrates, is greater in cropped than in uncropped soils and that the growing plant is an important agent in stimulating that activity.(33). It is quite possible, therefore, that the production of carbon dioxide, as well as the absorption of electrolytes by the plant, plays a part in raising the pH value of the soil.

Robertson (45) found a remarkable similarity in the effects of plant growth and carbon dioxide on the acidity of similar samples of soils to those examined here. Each soil was made up to 20 per cent. moisture content and, in addition to small samples used, as described above, for experiments with peas, samples of 100 g. were lightly packed in glass tubes ($\frac{3}{4}$" diam.). One set of tubes was connected in series to a supply of carbon dioxide and a second set was left open to the air. The tubes containing different soils were connected in the same order in each set and a wash bottle was placed before each tube to main-

tain a constant moisture content of the soil throughout. Carbon dioxide and air were drawn through the two sets respectively at the same rate. At intervals, small samples of soil were removed from both ends of each tube and mixed for pH determinations. He found that the results obtained from the "CO_2-treated" samples were not affected by passing a rapid current of air through the suspension immediately before measuring the acidity. When carbon-dioxide was passed through aqueous suspensions of the original soils, the acidity gradually increased, but decreased again, on aeration, to values corresponding closely to those obtained by the passage of carbon dioxide through the moist soil.

The effect of the carbon dioxide was in general not so great as that of the plant, but the results were not strictly comparable since the former was measured at a much lower temperature (12-14°C.). There was usually an actual increase in the pH values of the "CO_2-treated" samples, while the values for the "air-treated" samples remained comparatively constant, indicating that biological activity had probably been small.

In order to examine this question more fully, a fresh series of experiments was carried out with soils G, B, L and a mixture of D and L (which are both garden soils). These soils were selected because of the range in physical texture and concentration of water soluble constituents which they afforded. They might all be described as fairly common types of fertile soil, slightly acid but by no means seriously unsaturated, and containing normal amounts of organic matter for similar soils in this area.

(iii) Effects associated with changes in soil acidity. The investigation consisted of three sets of experiments in which/

which were examined (a) the effect of peas (b) the effect of oats and (c) the effect of carbon dioxide on the chemical properties of the soil. It was necessary to start with quantities of soil which were large enough to yield sufficient volumes of extract for analysis, and, in all the experiments, a bulk sample was brought to a suitable moisture content, allowed to stand overnight and subdivided into portions corresponding to 90 g. air dry material. In the plant experiments, these small samples were lightly packed into beakers and germinated seeds were planted a short distance below the surface. Nine peas or 30 oat grains were planted in each beaker. After being kept at a reasonably constant moisture content and temperature in a glass frame for a certain period, the seedlings were carefully removed from the beakers, the soil adhering to the roots and the rest of the soil in the beaker were washed into a bottle with a volume of water amounting to 450 cc., and the suspension was shaken for 30-40 minutes. A portion of the suspension was taken for a pH measurement and the remainder was filtered and, if necessary, refiltered to get a clear extract. Extracts were similarly obtained from the soil in control beakers and every test was carried out in duplicate. In other words, duplicate determinations were not made on aliquot portions of the same extract but on duplicate extracts. In the experiments with carbon dioxide, the moist soil was packed into two sets of glass tubes about 1" diameter and 8 to 10" long. A wash bottle was connected between every two tubes to prevent the soil from drying during the passage of gas. Air was drawn through a soda lime tube, a wash bottle and then through one set of tubes by means of a filter pump. A steady stream of carbon dioxide from a cylinder was washed first in bicarbonate and then in water and allowed to pass through the second set of tubes. After the specified/

specified periods of treatment, a tube was removed from each set and extracts were prepared as described above. These experiments were not carried out in duplicate but several checks on the results were obtained in numerous preliminary trials.

In view of the possibility of rapid changes taking place in the composition of these extracts, every effort was made to get the analyses started as soon as the filtrate was available. The acidity of the suspension was measured at once; then a portion of the filtrate was titrated with $^{N}/_{20}$ HCl for the bicarbonate estimation - the end point was taken with methyl red at boiling point - and set aside for the calcium determination (appendix I). Another portion of the filtrate was used for a rapid measurement of electrical conductivity and then a portion was taken for the determination of nitrate (appendix VI). Usually, the first stage of the analysis was completed within 24 hours of the preparation of the extract, and, when that was not practicable, a few drops of xylol were added to each bottle and seemed to prevent the development of any organisms.

The results are summarised in tables 1, 2 and 3. The most striking feature of the results is again the reduction in acidity invariably produced by the growing plants. The effect of the pea seedlings has been much greater than that of the oat seedlings , but since some months elapsed between the two sets of experiments and the soils were not exactly the same, a close comparison of the results cannot be attempted. With respect to the action of carbon dioxide on soil acidity, the effect is small in the case of soil G, it is towards an increase in acidity in the case of soil B and, in the case of soil DL, it amounts to a fairly large decrease in acidity. The results are, therefore, not so regular and conclusive as those obtained by Robertson. This might be due to the differences of technique, for/

TABLE 1

Influence of pea seedlings on soil properties.

Soil	No.	Time in days	pH	H $\times 10^{-6}$	C gemmhos	Milli-equivalents per 100g. soil $\times$ 1000 HCO_3'	NO_3'	$Ca^{\cdot\cdot}$	"Total"	A[1]
G	0	-	6.42	.380	104	140	4	345	489	349
control	1,2	5	6.45	.355	112	68	68	295	431	363
"	3,4	9	6.39	.407	120	77	105	337	519	442
"	5,6	13	6.34	.457	126	48	127	352	527	479
"	7,8	17	6.31	.490	143	53	207	438	698	645
planted	9,10	5	6.56	.275	120	195	41	320	556	361
"	11,12	9	6.82	.151	159	355	36	295	686	331
"	13,14	13	6.87	.135	184	365	90	247	702	337
"	15,16	17	6.85	.141	228	335	267	335	939	602
B	0	-	5.56	2.75	200	100	10	488	598	498
control	1,2	5	5.81	1.55	215	32	22	482	526	504
"	3,4	9	5.81	1.55	196	49	27	462	538	489
"	5,6	13	5.80	1.58	200	26	40	462	528	502
"	7,8	17	5.78	1.66	205	27	41	474	542	515
planted	9,10	5	5.82	1.51	200	78	18	460	556	478
"	11,12	9	6.07	0.85	182	125	16	398	539	414
"	13,14	13	6.21	0.62	190	83	23	340	446	363
"	15,16	17	6.23	0.59	205	100	40	360	500	400
D.L.	0	-	6.40	.398	280	175	64	670	909	734
control	1,2	5	6.59	.257	300	110	179	730	1019	909
"	3,4	9	6.50	.316	348	72	318	911	1301	1229
"	5,6	13	6.33	.468	365	54	446	987	1487	1433
"	7,8	17	6.28	.525	400	59	547	1135	1741	1682
planted	9,10	5	6.62	.240	290	213	102	723	1038	825
"	11,12	9	6.55	.282	273	191	197	763	1151	960
"	13,14	13	6.59	.257	330	172	404	806	1382	1210
"	15,16	17	6.36	.437	455	137	897	1195	2229	2092

1. A denotes (NO_3' + $Ca^{\cdot\cdot}$) or ("Total" - HCO_3')

TABLE 2

Influence of oat seedlings on soil properties.

Soil	No.	Time in days	pH	H $\times 10^{-6}$	C gemmhos	Milli-equivalents per 100g. soil $\times$ 1000 HCO_3'	NO_3'	$Ca^{\cdot\cdot}$	"Total"	A[1]
G	0	-	6.31	.490	102	113	24	-	-	-
control	1,2	5	6.48	.331	104	106	41	270	417	311
"	3,4	10	6.34	.457	113	104	108	290	502	398
"	5,6	13	6.29	.513	123	96	143	350	589	493
"	7,8	16	6.15	.708	138	106	209	440	755	649
planted	9,10	5	6.46	.347	97	115	24	270	409	294
"	11,12	10	6.33	.468	69	153	19	270	442	289
"	13,14	13	6.31	.490	70	159	17	280	456	297
"	15,16	16	6.37	.427	65	123	22	260	405	282
B	0	-	5.67	2.14	198	78	21	-	-	-
control	1,2	5	5.70	2.00	191	69	18	460	547	478
"	3,4	10	5.72	1.91	199	86	22	460	568	482
"	5,6	13	5.70	2.00	190	88	27	470	585	497
"	7,8	16	5.67	2.14	215	74	27	530	631	557
planted	9,10	5	5.76	1.74	187	75	13	450	538	463
"	11,12	10	5.71	1.95	112	108	11	330	449	341
"	13,14	13	5.75	1.78	118	101	11	330	442	341
"	15,16	16	5.75	1.78	117	96	11	340	447	351
L	0	-	6.61	.246	450	83	164	-	-	-
control	1,2	5	6.59	.257	480	95	293	1490	1878	1783
"	3,4	10	6.48	.331	500	91	567	1670	2328	2237
"	5,6	13	6.49	.324	520	101	563	1740	2404	2303
"	7,8	16	6.48	.331	505	95	590	1720	2405	2310
planted	9,10	5	6.58	.263	450	105	239	1460	1804	1699
"	11,12	10	6.69	.204	325	108	63	1210	1381	1273
"	13,14	13	6.69	.204	350	140	98	1270	1508	1368
"	15,16	16	6.70	.200	343	125	96	1240	1461	1336

1. A denotes (NO_3' + $Ca^{\cdot\cdot}$) or ("Total" - HCO_3')

for, although he found that the aerating of the suspensions for a few minutes before making the pH measurements had no influence upon the results, the suspensions were not shaken for half an hour as in this case, and might not have reached equilibrium. The evidence shows, however, that the acidity of an acid soil may be slightly decreased by the action of carbon dioxide.

The hydrogen ion concentrations of the soil suspension, given in addition to the pH values in the table, emphasise the actual changes in acidity that have occurred. The absolute values of the ionic hydrogen associated with the soils are, of course, fantastically low in spite of their importance and do not influence the balance of ions in solution to any extent. In view, also, of what has been said on experimental error, it is perhaps advisable to adhere to the pH figures in the discussion of the results. The bulk of the cations in solution probably consists of calcium, but the large discrepancy throughout between the +ve and -ve ions makes it obvious that nitrate and bicarbonate contribute a much smaller proportion of the total anions in solution. The concentration of phosphate ion in soil extracts is usually of the order of a few parts per million so that the rest of the anions is probably composed mainly of sulphate and chloride. These are not nearly so subject to fluctuation as the nitrate and bicarbonate and it is not unreasonable, therefore, to discuss the rapid changes which take place in terms of the ions whose concentrations have actually been measured. The figures for electrical conductivity bear a fairly close relationship to the "total ions" but they were taken, in the first instance, merely as checks and are liable to be influenced by the colloidal material in soil extracts.

To consider table 1 first, it will be observed that the young plants have brought about a remarkable increase in the concentration/

centration of bicarbonate in all three soils: they have reduced the nitrate concentration to begin with, but, by the end of 17 days growth, have presumably so stimulated biological activity that any nitrate absorbed by them has been made good by an increased production of nitrate, particularly in soils G and DL; there is an indication that the above remark might also apply to the effect upon soluble calcium for, although it is always less in presence of the plants, it tends to increase towards the end of the observations. Largely on account of the increase in bicarbonate, the "total" concentration of ions has actually been increased by the plants in the case of the two soils G and DL, and, in the case of soil B, the reduction is comparatively small; results which would, of course, be most unlikely to occur under the less favourable conditions for biochemical change in the field.

The results obtained with the oat seedlings (table 2) are rather different. The oats have increased the concentration of bicarbonate but to a much smaller extent than the peas, and the nitrification changes have not been able to maintain the concentrations of nitrate or calcium. The result has been that the plants have effected a considerable reduction in the concentration of "total" ions, a fact which is confirmed by the conductivity values for the extracts.

In some respects the effect of carbon dioxide is similar to that of the plants. For example, the concentration of bicarbonate is practically doubled and the concentration of calcium is definitely increased. On the other hand, the carbon dioxide has effected a very marked restraint on the chain of processes connected with nitrate production, the final concentrations of nitrate being less than the original (presumably due to the assimilation of nitrate previously present).

The/

TABLE 3

Influence of carbon dioxide on soil properties.

Soil	No.	Time in days	pH	H $\times 10^{-6}$	C gemmhos	Milli-equivalents per 100g. soil $\times$ 1000 HCO_3'	NO_3'	$Ca^{\cdot\cdot}$	"Total"	A^1
G	0	-	6.35	.446	104	98	24	321	443	345
air	1	5	6.54	.288	105	98	36	279	413	315
"	2	7	6.53	.295	100	100	44	281	425	325
CO_2	3	5	6.57	.269	99	185	4	299	488	303
"	4	7	6.56	.275	104	203	4	315	522	319
B	0	-	5.71	2.46	192	53	24	479	556	503
air	1	5	5.81	1.55	188	65	18	450	533	468
"	2	7	5.87	1.35	195	73	21	459	553	480
CO_2	3	5	5.77	1.70	190	108	4	498	610	502
"	4	7	5.81	1.55	200	123	5	480	608	485
DL	0	-	6.49	.324	270	158	13	710	881	723
air	1	1	6.48	.331	270	150	21	668	839	689
"	2	2	6.59	.257	270	140	113	650	903	763
"	3	6	6.67	.214	280	123	199	696	1018	895
"	4	7	6.62	.240	300	133	263	755	1151	1018
CO_2	5	1	6.46	.347	305?	218	16	836	1070	852
"	6	2	6.57	.269	280	250	40	760	1050	800
"	7	6	6.83	.148	270	365	9	728	1102	737
"	8	7	6.79	.162	280	388	8	708	1104	716

1. A denotes (NO_3' + $Ca^{\cdot\cdot}$) or ("Total" - HCO_3')

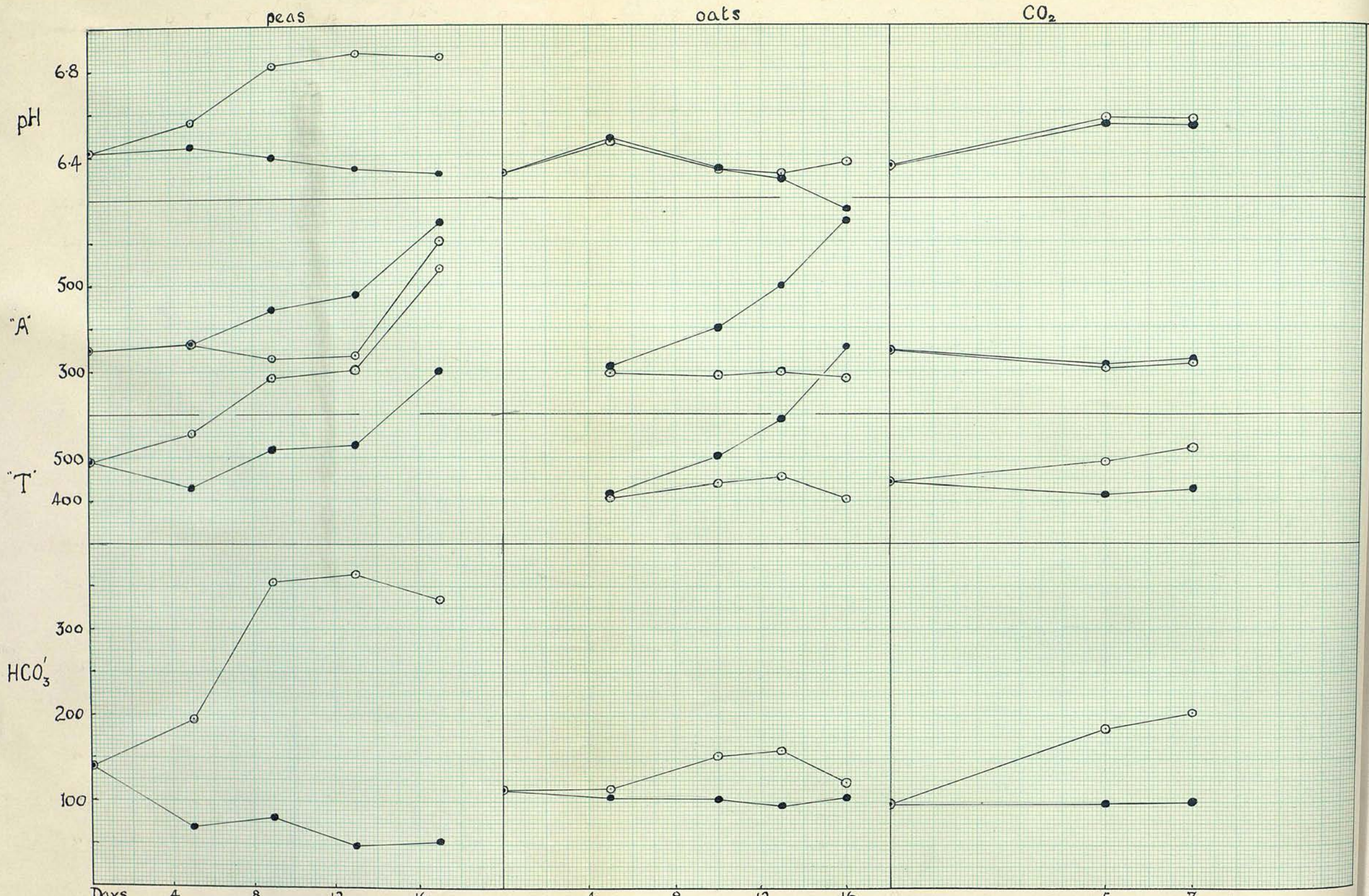

peas
oats
CO_2
pH
6·8
6·4
"A"
500
300
"T"
500
400
HCO_3'
300
200
100
Days
4
8
12
16
4
8
12
16
5
7
planted
control
Carbon dioxide
air

Fig. 13. Soil G.

The general relationships existing between acidity and the concentrations of (nitrate + calcium), bicarbonate and "total" ions are shown graphically in figs. 13, 14, 15.

If the changes in soil acidity were associated mainly with the concentration of electrolytes in the soil solution it might have been expected that the oat plants would have produced much greater effects than the peas, but the reverse has been the case. The idea, in the case of peas, that the reduction in acidity, in spite of the increase in soluble salts, may have been due to the fact that the increase is mainly accounted for by bicarbonates, receives a good deal of support from the figures for ("total" - HCO_3'). These are, with the exception of the last value for soil DL, consistently lowered by the plants. The same thing is to be observed in the case of the carbon dioxide treatment, there being an almost perfect negative correlation between pH and ("total" - HCO_3') or, what amounts to the same thing here, (NO_3' + Ca''). This means that the association between acidity and soluble salts becomes much closer when the effect of bicarbonate is eliminated, or, in other words, that the presence and change in concentration of bicarbonate is a most important factor in determining soil acidity. Only a rough approximation is possible with these results, for, even if the relative effects of the bicarbonate and this "total" less bicarbonate were known, there would still remain the contribution of the various other ions to be considered. It must be admitted, however, that this explanation is not entirely satisfactory when applied to the results for oats.

The effect of the plant is evidently determined by the absorption of ions, the excretion of carbon dioxide by the roots, and the stimulation of biological activity with the liberation of more carbon dioxide and free ions. There may be other factors and/

and the complexity of the problem obviously makes it futile to attempt any more precise analysis of the available data.

DISCUSSION OF RESULTS.

The results described above have been obtained under extremely artificial conditions, and although they probably give a better indication of what might happen under field conditions than the observations made with culture solutions or with sand cultures, they are subject to a number of important limitations. As has already been pointed out in connection with pot experiments, the handling of a soil in the laboratory, especially after remoistening, is liable to bring about very important changes in its properties. These changes are bound to be more rapid and pronounced than in pot experiments, as a result of holding the soil at an almost constant moisture content and temperature, both ideally suited for biochemical changes. Furthermore, the very extensive ramification of the roots through the small mass of soil, the extremely quick growth of the seedlings and the lack of drainage would all contribute in the direction of exaggerating similar effects in the field. It is probable, in fact, that the effects produced in a few days in such labroatory experiments are greater than those which might be produced over much longer periods under less favourable conditions. Nevertheless, an opportunity is given for all the possible chemical and biological changes and interactions in the soil to take place, and the observations concern the net result of these phenomena. Under field conditions, the succession of changes might be broken by climatic factors and indeed some of them might never occur, but it is felt that the laboratory results represent what could happen during/

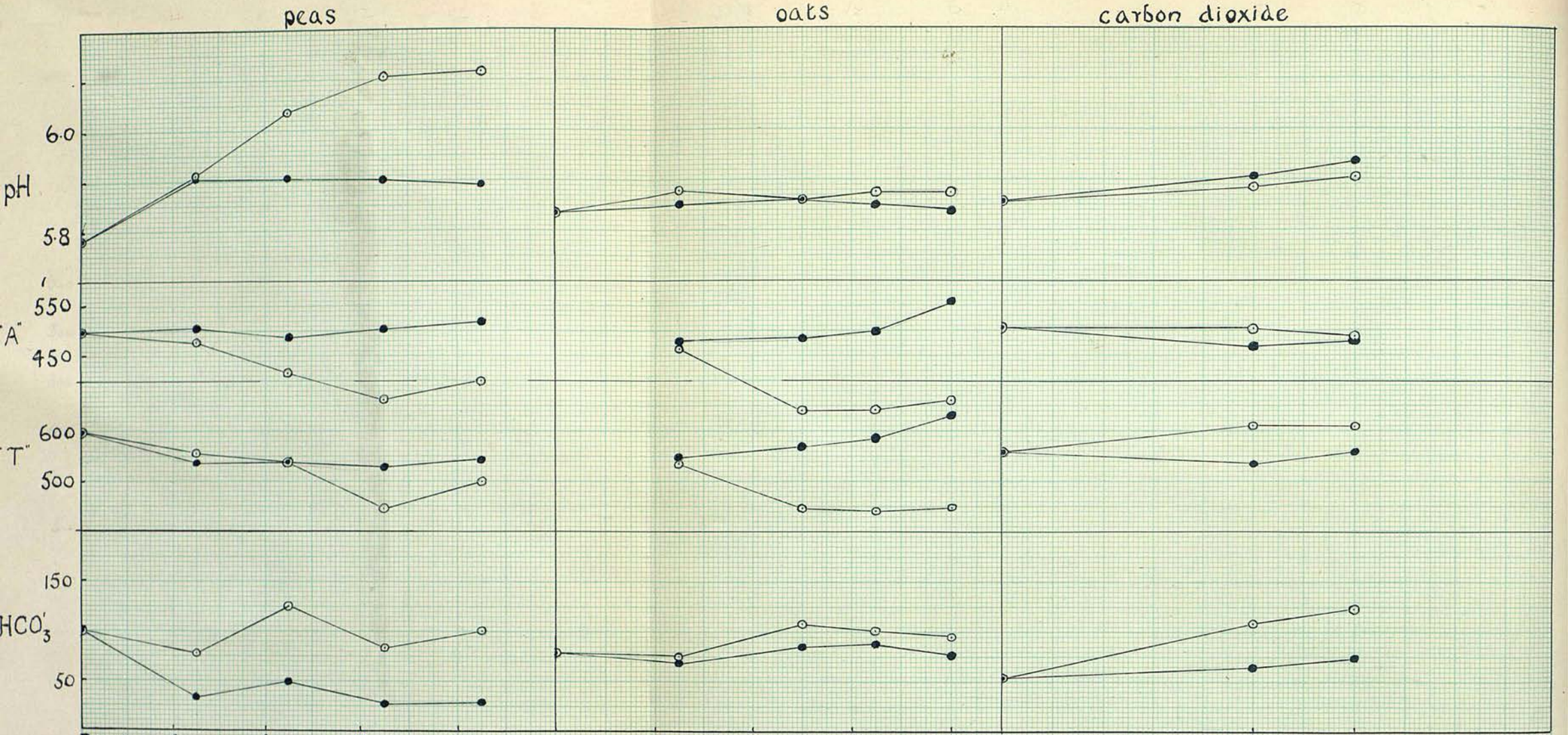

peas
oats
carbon dioxide
pH
6·0
5·8
'A'
550
450
'T'
600
500
HCO'3
150
50
Days
4
8
12
16
4
8
12
16
5
7
planted
control
CO2
air

Fig. 14. Soil B.

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during a period of suitable weather in a well cultivated soil supporting a vigorous crop. The fluctuations due to seasonal changes in moisture and temperature would then be small compared to the variations due to the crop. Such a state of affairs seldom exists during the growing season and the effect of the crop alone cannot be determined. Consequently, the laboratory experiment is the only method of approaching the problem.

It has been shown that the variations in soil acidity in the field are definitely modified by the growing plant and these observations have been supported by extended pot experiments where normal climatic factors were disturbed only by occasional necessity for watering. This influence of the plant seemed to be related, in some fairly close manner, to the quantities of water soluble material in the soil - presumably due to the absorption of plant nutrients. That is as far as the field and pot experiments have been carried. These results have been supported by numerous laboratory experiments in which, with moisture and temperature effects under control, it has been repeatedly demonstrated that the young plant actually effects a relative decrease in the acidity of the soil. The affect is not constant for different soils or different plants, but it varies only in degree. The earlier hypothesis, that the ability of the growing plant to reduce the acidity of the soil was due to the absorption of cations with a consequent decrease in the exchange acidity in aqueous suspension of the soil, has not been proved by analyses of the soil extracts. These show that the soil carrying plants frequently contains larger quantities of soluble constituents than the same soil without plants, and yet it is less acid. It is fairly obvious, therefore, that the fluctuations in soil acidity are not associated directly with changes in the concentration of the soil extract when growing plants are present. Trofimov (69)/

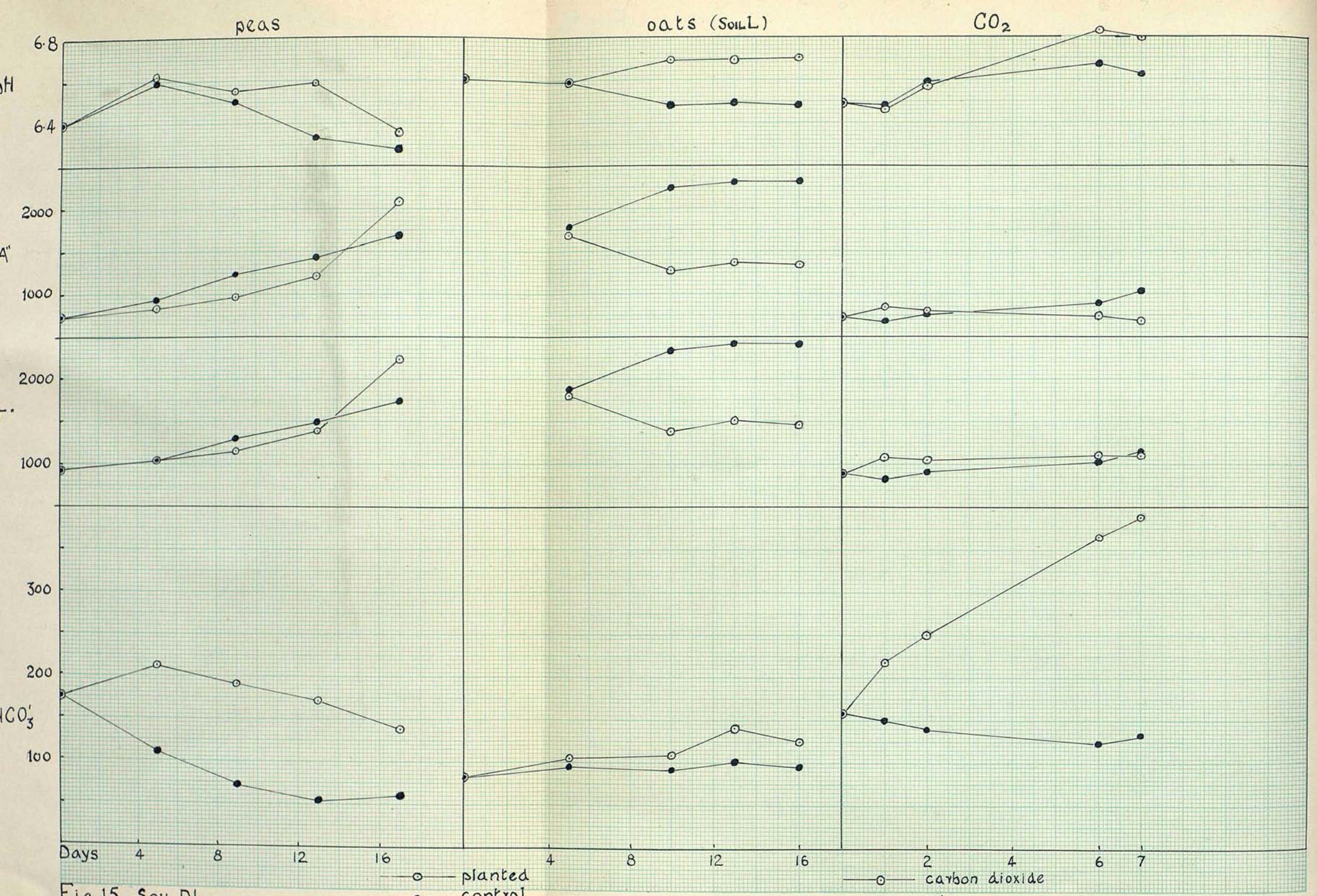

Fig. 15. Soil DL.

(69) has demonstrated with considerable success that the concentration of the soil solution is so closely related to the acidity that the former may be calculated from pH figures for the soil in water and calcium chloride solution. His equation is derived from a direct application of the rules governing the exchange of hydrogen and calcium $\frac{[C_a]}{[H]^2} = K$ and is suitably expressed in the form $pH = pH_1 - \log(C_o - C_1)$ where pH = acidity of the soil in N. $CaCl_2$; C_o = concentration of soil solution; C_1 = concentration of salt added to soil. This, of course, takes no account of possible changes in the relative proportions of the anions present, which may alter the buffer system of the soil. The displacement of other anions by bicarbonate would tend to decrease the acidity of an acid solution, for example.

In common with the culture solution results of Hoagland (23) which showed that nitrate was absorbed very rapidly from acid solutions and replaced by bicarbonate, the plants tend to reduce the concentration of nitrate, at least to begin with, and produce a large increase in the concentration of bicarbonate. These observations are also in agreement with many other investigations along different lines. Thomas (67), for example, has collected a mass of evidence, up to 1930, showing that there is an increase in the production of carbon dioxide and bicarbonates in the soil due to the presence of growing plants, particularly during their most active period of development. In more recent investigations, Starkey (61) has shown that the abundance and activity of micro-organisms and the production of carbon dioxide and nitrates are greater in the neighbourhood of plant roots than at a distance from them; Wurmbach (72) has found that soil "respiration" is markedly increased by plant growth; Sabinin (49) has demonstrated that the assimilation and respiration processes of the plant roots are initiated by the exchange of hydrogen and bicarbonate/

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bicarbonate, absorbed on the cell membrane, for nitrate and phosphate.

There is still considerable doubt as to the actual proportions of carbon dioxide produced by root respiration and by microbiological activity, but the matter is unimportant so far as the present investigation is concerned. The interesting point is that one effect of the growing plant is to produce a substantial increase, directly or indirectly, in the concentration of bicarbonate in the soil. How this bicarbonate may influence the acidity of the soil, to the extent of raising its pH value in spite of an increase in the total soluble salts, is demonstrated in several of the laboratory experiments. It is quite obvious that the part played by bicarbonate must be considered in any work designed to test the association between the acidity of the soil and its content of electrolytes.

Attempts to examine more closely the actual effect of carbon dioxide on the properties of the soil, by passing a current of the gas through moist soils, have not met with unqualified success. They show that the carbon dioxide may reduce the acidity of slightly acid soils as do plants, but the comparison cannot be carried much further. The passage of carbon dioxide may increase the quantity of soluble calcium in the soil, as was also shown by Stewart (62), but continued treatment is so drastic that the nitrification processes are practically stopped and the results are no longer comparable to those obtained with plants. The conditions, in fact, become similar to those in the culture solution studies made by Hoagland (22) and Theron (66), in which decreases in acidity were due almost entirely to a displacement of nitrate by bicarbonate. It seems, however, that the variation in concentration of bicarbonate is not solely responsible for acidity changes in soils for, in spite of the relatively large increase/

increase in bicarbonate and the relatively small change in "total" ions which are effected by carbon dioxide, the decrease in soil acidity is small. It seems as if there are other factors at work when the question of a growing plant is introduced, a probability that is supported by the minor differences in the effects produced by different species. The unequal rates of absorption of calcium, for example, have still to be definitely correlated with the various equilibria which are possible with different plants and different sets of nutrient conditions. It can confidently be stated, from the results of the investigations, that variations in soil acidity are related to changes in the concentration and composition of the soil solution, both of which may be affected by plant growth; but in view of the highly controversial nature of the available evidence on the feeding power of plants, it is not possible to amplify the statement meantime without considerable reservation.

It is perhaps difficult to appreciate the full practical importance of the question at this juncture. Only a limited amount of attention has been given to the effects of different degrees of acidity on the crop. The effect on the yield and the nitrogen content of oats is discussed in the next section (pp. 109-111) where it is shown that with a normal fertile slightly acid soil, varying degrees of acidity are, within limits, of secondary importance. Results of a similar character have been reported by Smith and Robertson (59) in connection with the yield and the calcium and sulphur content of various crops, but they have also shown that these results are not applicable to a soil which is strongly acid and seriously unsaturated. Photographs 1 and 2, for example, demonstrate the remarkable differences, effected by changes in acidity, between the normal soil/

soil B and soil W, which is seriously acid. But the pH figure only reflects other characteristics of the soil and differences which are commonly associated with degrees of acidity are often much better explained on the basis of unsaturation or even of exchangeable calcium. In another investigation, it has been shown by Robertson and Smith (46) that the acidity of potato tubers is apparently independent of environment and is not influenced by large variations in soil acidity.

These observations tend to minimise the degree of importance that is so commonly attached to soil acidity measurements, but there is another aspect of the question which requires to be considered. The growing plant is in very intimate contact with the soil particles and the changes which proceed during the absorption of nutrients have still to be established. The ultimate results may be measured, but the mechanism of the process is still quite obscure. It is extremely probable that what is usually called soil fertility is determined more by the interchange of ions between plant and soil and the symbiosis of plant and micro-organism and the chamical reactions thereby stimulated or inhibited than by any possible expression of the properties of the soil at a particular time. It is also certain that the hydrogen ion plays an important part in these changes, so that a continued study of its particular behaviour, and the consequences, will at least assist in reducing the present confusion and stating the problem in its simplest terms. Until this is done, the soil chemist must continue to use the system of more or less arbitrary tests in the assessment of soil fertility and manurial requirement.

SUMMARY.

SUMMARY.

A comprehensive examination has been made of the experimental errors associated with the sampling of soils in field and pot experiments and with the subsequent laboratory technique. It has been shown that the field error is usually more than double the laboratory error and that the latter may be of the order 10 per cent. in such determinations as are commonly made in soil analysis. Examples have been given in repsect of pH measurements in particular in order that the results described later may be critically assessed,

It has been shown, as a result of field and plot experiments extending over six years, thatthe acidity of slightly acid soils may fluctuate considerably during the growing season, that these fluctuations are modified by the growing plant and are related to the concentration of the soil solution as estimated by 1 : 5 soil in water extracts.

It has been further demonstrated that these observations may be confirmed in pot experiments and also in laboratory experiments where climatic factors do not play a part.

It has been established, by laboratory experiments, that the growing plant reduces the acidity of an acid soil and that this action is general for different species and different soils; that the effect is related to the quantity of soluble material in the soil but not necessarily to the concentration; that the displacement of nitrate by bicarbonate, which occurs during plant growth, is a very important factor in the results.

Experiments have also been made to compare the effects of the growing plant and carbon dioxide, and it has also been shown that carbon dioxide may reduce soil acidity but that the effect is small for the comparatively large increase in bicarbonate/

bonate ion. The relative effects of the bicarbonate and other ions have been discussed and certain views on the practical importance of the results have been submitted.

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APPENDIX I

A series of 23 duplicate determinations of calcium in 1 : 5 soil - water extracts.

The method of analysis consisted in adding about 1 cc. 2N. HCl to 200 cc. of extract, evaporating to about 100 cc., filtering off any material in suspension and washing 3 times with hot water. To the filtrate at boiling point were slowly added 10-20 cc. of a saturated solution of ammonium oxalate (also at boiling point) and then dilute ammonia in moderate excess. The mixture was boiled for 2 or 3 minutes and then placed on a hot plate overnight. The precipitate was filtered off, washed 3 times with warm dilute ammonia, dissolved in 15-20 cc. 4N. H_2SO_4 and titrated at 70°C. with N/20 $KMnO_4$. The titration figures varied for different soils from about 2 to 14 cc.

The standard deviation σ_w, where w represents the absolute difference between duplicate determinations, was 0.22. There were indications that the difference between two results depended upon the magnitude of the calcium present in the sample, but insufficient data were available for a precise examination. The general mean, $\overline{w}$, was 0.185.

It may be concluded that a difference greater than about 0.4 is most unlikely in duplicate determinations by this method - actually there was one in the 23 examples. The standard deviation of 0.22 represents approximately 0.027 mgm. equiv. of calcium in 100 g. soil and, since the average value is about 0.7, duplicate results may be said to agree within 7 per cent.

APPENDIX II.

Computation of the Errors involved in estimating the degree of infestation of Heterodera schachtii in Soils.

TECHNIQUE.

Field. Generally speaking, a strip about 5 yards wide, and running through the centre of a patch previously known to be affected with disease, was selected for examination. The strip was subdivided into lengths varying from 10 to 15 yards so that each plot sampled has an area of from 50 to 75 sq. yards. At least ten borings to plough depth were made at intervals over each plot, a soil auger being employed for the purpose. Except in two cases which will be discussed later, the borings were mixed to give a composite sample. This method was devised to get a representative sample of soil to plough depth and overcome the difficulty due to the fact that some centres had been ploughed whilst others remained unploughed at the time of sampling.

Laboratory. The composite samples were spread out in the laboratory and allowed to reach an air-dry condition. They were then broken up and the material passing through a 2 mm. sieve was employed in the examinations. For cyst counts, ten samples of 10 c.c. were taken, by the usual method of quartering, from each composite sample. The cysts were removed from the soil by the method described by Morgan (3). The sample of soil was placed in a Stohmann shaking bottle or a standard flask, and shaken with about 200 c.c. of water for 4 or 5 minutes. The flask was then filled up with water and allowed to stand until the cysts had floated with undecayed vegetable matter to the surface. The floating material was then thrown on to a filter paper and the cysts counted under a low power lens. A number of cysts adhere to stones and fragments greater than 2 mm. and are therefore lost in the above method. To estimate the loss incurred, the material

greater/

greater than 2 mm. in diameter was examined in ten cases for peat soils, and in four cases for sandy soils. The greatest loss was 3.3 per cent. for a peat soil and 1.2 per cent. for a sandy soil, the respective averages being 2.5 per cent. and 0.9 per cent. As will be seen from the tables which follow, these figures, which were fairly constant for the two soil types, are scarcely worthy of serious consideration.

SOURCES OF ERROR.

The counts made as described are liable to two important sources of error namely, (a) the field error due to taking ten borings to form a composite sample representative of a plot, and (b) the laboratory error due to the sampling and counting of the sieved air dried soil. An attempt has been made to express those errors in the form of percentage standard error.

Laboratory error. Sufficient data are available to form a good estimate of the standard error due to laboratory sampling and counting, for over 90 samples, yielding about 900 counts, were carefully examined. Table I is typical of a series of samples and is set out at length to show the variation found in the individual counts of a sample and the method of arriving at the standard error of each mean.

The results of every sample have been subjected to the same treatment to get an estimate of the standard error due to sampling and counting in the laboratory. For each series of plots, the percentage error has been averaged[1] for those counts of more than ten cysts per 10 c.c. of soil. That limit has been chosen arbitrarily in order to exclude those samples taken from a strip running beyond an area actually infested and those samples in which the number of cysts was so small as to make the standard error/

[1] Since only an estimate of the error was desired the arithmetic mean has been taken.

Table I.

The cyst counts in a series of ten composite samples and the estimation of the laboratory standard error.

Sample 120	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
	32	44	41	57	47	41	48	40	54	35
	34	51	42	48	42	36	50	52	28	25
	44	43	30	49	53	48	54	61	52	28
	31	44	29	42	50	36	48	49	41	37
	35	39	39	42	47	44	59	65	48	42
	31	39	39	49	57	44	68	59	40	30
	21	44	33	37	58	42	50	64	58	30
	40	42	35	52	45	30	51	55	42	29
	33	50	37	45	40	43	57	48	64	30
	32	49	50	52	52	34	46	40	43	27
$\bar{X}$	33·3	44·5	37·5	47·3	49·1	39·8	53·1	53·3	47·0	31·3
$S(X-\bar{X})^2$	328·0	162·0	348·0	312·0	325·0	181·0	399·0	748·0	874·0	240·0
σ^2	36·4	18·0	38·7	34·7	36·1	20·1	44·3	83·1	97·1	26·7
$\sigma/\sqrt{10}$	1·91	1·34	1·97	1·86	1·90	1·42	2·10	2·88	3·12	1·63
% error	5·73	3·02	5·25	3·94	3·87	3·56	3·96	5·41	6·63	5·22
χ^2	9·85	3·64	9·28	6·60	6·62	4·55	7·52	14·04	18·60	7·67

Average percentage error = 4·66.

$\bar{X}$ = the arithmetic mean of each set of ten individual counts.

If X = any one count, $(X-\bar{X})$ is the deviation from the mean.

$S(X-\bar{X})^2$ = sum of the squares of the deviations.

σ^2 = the variance = $\dfrac{S(X-\bar{X})^2}{n-1}$ for small samples, when n equals the number of counts (10).

σ = the standard deviation and $\sigma/\sqrt{10}$ = the standard error of the mean.

$\chi^2 = \dfrac{S(X-\bar{X})^2}{X}$.

Table II.

The percentage standard errors due to laboratory technique and the indices of dispersion.

Soil type	Series	Number of samples	Average standard error per cent.	Sn	χ^2
Peaty sand	23–32	10	7·34	90	93·11
	124 *A*–124 *E*	4	6·82	36	29·21
	66–75	3	6·44	27	30·92
	Weighted mean		7·06		
Peat	46–55	4	6·22	36	34·01
	113 *A*–113 *C*	3	7·89	27	42·44
	11–20	10	4·31	90	61·44
	111 *A*–111 *D*	4	9·02	36	40·82
	112 *A*–112 *D*	3	8·49	27	39·67
	6–10	2	5·49	18	18·83
	56–65	10	5·86	90	84·01
	120 *A*–120 *J*	10	4·66	90	88·37
	101–110	10	5·30	90	91·61
	Weighted mean		5·76		
	Total	73	—	657	654·44

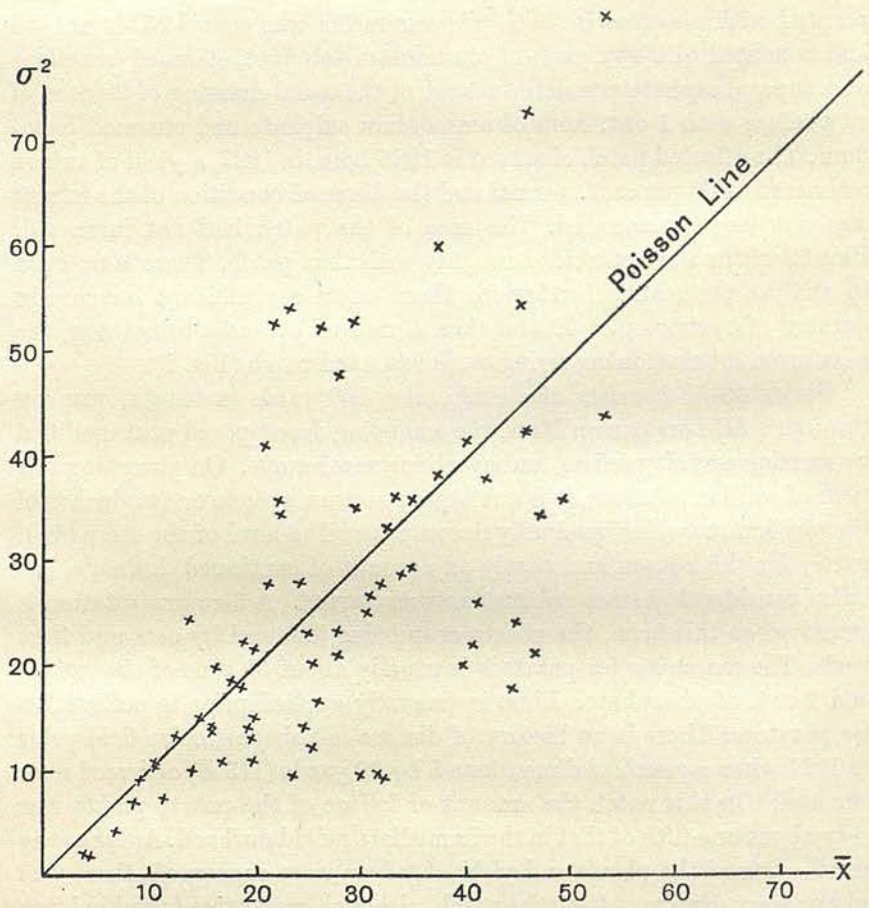

Fig. 1. Diagram representing association between $\bar{X}$ and σ^2 for cyst counts.

error quite abnormal. Table II gives a summary of the results.

The figures in Table II show that when sandy soils are considered the standard error due to laboratory technique is of the order of 7.1 per cent., whilst for the peat soils, which incidentally were found to have much greater counts, the error is in the neighbourhood of 5.8 per cent. That laboratory error appears to be large, but a more careful consideration of the position indicates that it is what might be expected. If a particle is taken from the soil, the chance of its being a cyst is very small indeed: if, therefore, samples consisting of large numbers of particles are taken at random from a bulk sample, the numbers of cysts in those samples should be distributed according to a Poisson series. An agreement of the results with the theoretical distribution affords a test of the suitability of the technique, and they have been submitted to an analysis similar to that employed by Fisher, Thornton and Mackenzie (2). For all Poisson series the variance is numerically equal to the mean, and the index of dispersion, $\chi^2 = \frac{S(X - \bar{X})^2}{\bar{X}}$, is distributed in a known manner so that, for every value it assumes, there is a corresponding value P representing the probability that χ^2 will be exceeded by chance. In other words, it is possible to test the agreement between the results obtained and those expected. As a first approximation σ^2 was plotted against $\bar{X}$ and Fig. 1 shows that the majority of the points lie fairly closely to the line representing a true Poisson series. With a single sample of ten counts the range of variation of χ^2 is too great to be of much value, but the sum of any number of quantities χ^2 is distributed in the χ^2 distribution, and it is therefore at least possible to test if the variability from expectation is normal. The values of χ^2, calculated as in Table I for each sample of ten counts, have been summed for each series of samples, Table II. In this

case/

case Sn is equal to the sum of the various values of n for the separate samples, n being one less than the number of counts.

To test if the value 654.44 for χ^2 is normal for n = 657, use has been made of the fact that for such a large value for n, $\sqrt{2\chi^2}$ is approximately normally distributed about $\sqrt{2n - 1}$ with unit standard deviation. In this case

$$\sqrt{2\chi^2} = 36.18,$$
$$\sqrt{2n - 1} = \underline{36.24},$$
$$\text{Difference} = -.06.$$

The difference is much less than the standard deviation, so that the variability between parallel counts is quite normal.

Finally, values for χ^2 are set out in Table III at intervals alongside the expected values taken from a χ^2 table (1).

The agreement is very good. By taking eight groups the probability of obtaining a worse fit by chance from normal data is about .2, so that there is no significant deviation of the values from expectation. The analyses of the results seem to indicate, therefore, that most of the sets meet the conditions required by samples from the Poisson series and that, therefore, the technique was satisfactory and the mean value for each set of counts a reliable estimate of the number of cysts of Heterodera schachtii present in the soil sample. It follows that if the technique were perfect there would still be an inherent percentage standard error equal to $\sqrt{\frac{20.44}{10}} \times \frac{100}{20.44} = 6.99$ for the peaty sands and $\sqrt{\frac{32.18}{10}} \times \frac{100}{32.18} = 5.57$ for the peats, where 20.44 and 32.18 are the respective means when samples having counts less than 10 are omitted as previously. The values found compare very favourably with those calculated and may therefore be employed with some confidence as giving a measure of the laboratory error.

Field error. A large variation in the numbers of cysts from point to point on a plot is to be expected. To get figures

Table III.

Comparison of observed and expected distribution of χ^2.

χ^2	Expected	73 % expected m	Observed $m+x$	x^2/m
4·168	10	7·3	7	0·012
5·380	10	7·3	7	0·012
6·393	10	7·3	6	0·232
8·343	20	14·6	22	3·750
10·656	20	14·6	18	0·792
12·242	10	7·3	2	3·849
14·684	10	7·3	5	0·725
and over	10	7·3	6	0·232
Total	—	73·0	73	$\chi^2=9·604$ $P=0·2$

Table IV.

Variation in cyst content over two small plots and estimation of field errors.

Peaty sand plot 124 *C*			Peat plot 11*		
Sample	Mean of 10 counts (X)	$(X-\bar{X})^2$	Sample	Mean of 10 counts (X)	$(X-\bar{X})^2$
23	16·3	7·3	101	25·8	100·0
24	14·0	25·0	102	32·0	4·8
25	11·4	57·8	103	25·5	106·1
26	10·6	70·6	104	45·7	98·0
27	15·9	9·6	105	35·1	0·5
28	22·3	10·9	106	62·7	723·6
29	17·8	1·4	107	26·3	90·3
30	19·5	0·3	108	45·3	94·1
31	24·3	28·1	109	31·8	16·0
32	37·5	342·0	110	27·9	62·4
Total	189·6	553·0	—	358·1	1295·8
Mean $\bar{X}$ ...	18·96	—	—	35·8	—
Variance (σ^2)		61·4			143·9
Standard error of mean ($\sigma/\sqrt{10}$)		2·48			3·79
Percentage standard error ...		13·1			10·6
Percentage $\sigma_L/\sqrt{10}$		7·3			5·3
Percentage $\sigma_F/\sqrt{10}$		12·8			10·5
Percentage "total error" ...		14·7			11·7

* Samples 101–110 were taken 6 months later than composite sample 11.

for that variation in order to arrive at an estimate of the error involved in making a composite sample of ten borings, the following experiment was carried out. Ten samples were taken from each of the plots 1240 and 11, and examined separately. Ten counts were made for each sample. Table IV summarises the results.

The values 13.1 and 10.6 represent the percentage standard errors of the mean of a total of mn counts on n borings

$$= \sqrt{\frac{\sigma_L^2}{mn} + \frac{\sigma_F^2}{n}} \qquad \ldots\ldots\ldots(A),$$

where m = n = 10,

$\sigma_L / \sqrt{m}$ = the percentage standard deviation of the mean of m counts.

$\sigma_F / \sqrt{n}$ = the percentage standard deviation of the mean of n borings.

The value for $\sigma_F / \sqrt{n}$, representing the field error, has been calculated from equation (A).

Total error. The "total standard error" of the mean of m counts on a composite sample of n borings is then equal to $\sqrt{\frac{\sigma_L^2}{m} + \frac{\sigma_F^2}{n}}$ = 14.7 per cent. for the peaty sand and 11.7 per cent. for the peat.

The extent of those errors is due largely to the abnormal variations of samples 32 and 106 from the respective means. If those samples are excluded the "total standard errors" become respectively 11.1 per cent. and 9.1 per cent. The differences are considerable and serve to show how the large variation in infestation over a small plot may influence the results. It would be advisable to increase the number of borings taken to make the composite sample. For example, if the sample were obtained from 20 borings and 10 counts were made, the error of the mean would be reduced to about 9 per cent. for the peats. It is doubtful if increasing the number of borings beyond 20 would serve much useful purpose since a large increase in the size of the sample to be handled in the laboratory would make satisfactory manipulation difficult. As evidence of the fluctuations actually obtained/

obtained, the following results are of some interest. Plot 124C was sampled in the usual way, the mean of the ten counts being 19.7 compared with 18.96 for the ten samples 23-32, or 16.9 for the nine samples 23-31. Plot 46 overlapped plot 113B and the respective means were 23.5 and 25.0. In both cases, therefore, the difference between duplicates was quite insignificant, being less than the standard error.

Taking the standard error as about 14 per cent. for the peaty sands and 11 per cent. for the peats, it is now possible to review the results in greater detail.

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APPENDIX III

Variation in the composition of the displaced soil solution.

A certain amount of data is available on the effect of lime on the composition of the drainage water from tanks and lysimeters, and considerable attention has been given to the question of displacing the soil solution. It is unnecessary to do more than comment briefly upon the results because the literature has already been reviewed (4, 7).

The results obtained from different investigations on drainage water are not in absolute agreement, which is doubtless due to the varying depth and the different absorptive capacities of the soils employed. A survey of the data, however, indicates that liming usually increases the amount of magnesium removed from the soil, has no effect on the potassium, and little or no effect on the phosphate. Sometimes nitrification is increased, in which case more calcium is liberated, but with heavy soils that does not always occur, probably because their absorptive capacity is sufficiently high to prevent such a change in the soil solution as is necessary to stimulate nitrification.

The composition of the drainage water from a soil is not necessarily the same as that of the soil solution since leaching is accompanied by a succession of chemical equilibria. Consequently, an average value for a more or less prolonged period is obtained rather than the composition at any particular time. Furthermore, the investigation of the displaced soil solution involves a preliminary aeration and mixing of the soil, usually followed by an increase in the moisture content to the optimum for plant growth, and that treatment might be expected to produce more rapid changes than could occur in the field. The observations reported in this note were obtained in the course/

course of an examination of the soil solutions displaced at intervals from certain soils which had been treated with different amounts of dolomitic limestone, and indicate the nature of the early changes which may take place.

Experimental.

A silt loam (Group A, Table 1), and a sandy loam (Group B), having respectively 6 per cent. and 3 per cent. loss on ignition, were air dried and mixed with quantities of the ground limestone corresponding to half, once and twice the "lime requirement" as shown by the Truog test. The limestone contained about 50 per cent. calcium carbonate and 40 per cent. magnesium carbonate. The moisture contents were then made up to optimum and the soils placed in earthenware pots which were covered to reduce evaporation. Fresh samples of a silt loam (Group C), from two experiment plots, were allowed to dry to optimum moisture content, screened and stored in the same way. One plot had been limed twice in the rotation in 14 years, while the other had received no treatment during that period.

After definite intervals, noted in Table 1, each soil was screened and a portion packed unto a glass percolator. The soil solution was then displaced by a 0.5 per cent. solution of ammonium thiocyanate as described by Pierre (8) and calcium and magnesium were determined by standard methods. As has been stressed by other workers, the success of the displacement was found to rest in getting the soil uniformly packed, and so compacted as to allow the liquid to descend at a convenient speed. By packing about 30 g. at a time into a percolator 30 cm. long and capable of holding about 800-900 g. moist soil, it was found that over 30 per cent. of the original moisture could be obtained from the silt loam and about 20 per cent. from the sandy loam. For pH determinations, a soil suspension was taken rather than the displaced solution whose reaction changes appreciably on exposure/

exposure to the atmosphere. The quinhydrone electrode was employed with a Leeds and Northrup potentiometer. About 10 g. moist soil and a few decigrams of quinhydrone were mixed with 25 cc. distilled water, and allowed to stand for a few minutes before the reading was taken.

Results.

Burd and Martin (1) have discussed in detail the relation between the concentration of individual ions in the soil solution and the moisture content of the soil. The concentration of calcium is inversely proportional to the moisture content, but that relationship does not always hold for magnesium. The results for calcium and magnesium, submitted in Table 1, have been calculated to uniform moisture content but without introducing any serious error because the mean variation in moisture content in any group over the entire period was less than 2 per cent. All the results obtained with the silt loams have been calculated to 20 per cent. moisture content and those for the sandy loam to 8 per cent. They have been expressed as milligram equivalents per litre of displaced solution.

Discussion of results.

A consideration of the data for groups A and B reveals some interesting results. In both groups, storing under conditions favourable for nitrification processes has been as effective as the addition of dolomite in increasing the concentration of calcium and magnesium. Burd (2) has investigated the changes in the soil solution due to biological oxidation. The pH of his soil remained almost constant and the large increase in nitrate readily accounted for the increase in cations.

Table 1/

factor. Hissink (3) has shown that the rate of absorption of calcium carbonate depends chiefly upon the potential absorption of the soil and its degree of saturation with bases; but reserve calcium cabonate will play a big part in the mass action relationships and permanence of effect, while the nature of the soil solution will determine the amount of calcium which can come into solution and remain in solution. The nature of the soil solution is governed by such things as H-ion concentration and the presence of various anions which affect the solubility of the calcium. It will be observed that the pH of the lighter soil (B) has been altered more than that of the heavier soil (A) both by addition of limestone and by storage. Consequently, the variations in the concentration of calcium in the soil solution have been more pronounced in B than in A. Both groups show definitely that, while the addition of carbonate reduces the H-ion concentration of the soil, it does not necessarily increase the amount of calcium in solution.

MacIntire has carried out extensive lysimeter investigations with different liming materials and found (5, 6) that, after applications of dolomite in quantities equivalent to 8-100 tons of calcium oxide per acre, the outgo of calcium increased with increasing additions but that the outgo of magnesium did not. As already observed, the experimental conditions of the present investigation were widely different from those in lysimeter work, and the magnesium results reported here show a very decided and parallel increase due to the addition of dolomite as well as to biological activity. It is obvious that after 31 days there is still no evidence that the soil solutions have become saturated with magnesium as they have with calcium. The greater rate of change with the sandy loam compared with the silt loam is again clear, although the results for group B are not

quite/

quite complete. Unfortunately it was not found possible to effect another displacement to discover how much magnesium would ultimately go into solution, but it is interesting to observe that it has become roughly equivalent to the calcium.

That those results apply only to initial changes seems to be borne out by the data obtained from the experiment plot soil. The two applications of lime in the rotation have increased the quantity of calcium in the solution but reduced the quantity of magnesium. However, both cations would appear to be in a more available condition in the limed plot, as indicated by the respective increases brought about by 11 days storage under conditions suitable for biological activity. It is obvious that the concentration of the soil solution in the field is changed rapidly and to a considerable extent under the conditions described. It would seem, therefore, that the usual preparation of a soil for use in pot experiments must exert a marked influence upon the growth of the plant under examination (at least in the early stages) and introduce a factor of undoubted importance in assessing fertility and manurial requirements.

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APPENDIX IV

Observations on the Effect of Various Fertilisers on Soil Acidity.

The method employed in some preliminary work was to prepare a titration curve for fertiliser and soil by shaking 1 : 2.5 suspensions of soil in water with different quantities of the fertiliser overnight, and measuring the final pH values of the suspensions. A series of curves could then be drawn showing the effects of different substances on the same soil.

The three soils which have been examined are all from the College Experimental Farm but differ markedly in their properties. The very acid peat soil is from a thin covering on well weathered basic andesite at an elevation of about 1300 feet and the oven dry material has a loss on ignition of about 65 per cent. The clay loam occurs at the base of the hill within the area of an alluvial fan and contains about 20 per cent. each of clay, silt, fine sand and coarse sand by the international pipette method of analysis. Its loss on ignition is about 10 per cent. The sandy loam is from a glacial mound of sand and gravel, which at one time carried a plantation of trees and has certainly not been cultivated for 70 or 80 years. It is an extremely infertile soil with a high degree of unsaturation, contains more than 60 per cent. of fine and coarse sand and has a loss on ignition of about 11 per cent.

These soils were chosen as being representative of widely different types rather than of large areas in the East of Scotland.

In addition to calcium carbonate (precipitated) and calcium hydroxide (in solution), which have served as controls, the following fertilisers have been examined in the course of the investigation :- Superphosphate 18, 16 and 14 per cent., nitrochalk, ground mineral phosphate 34 and 26 per cent., calcium cyanamide/

TABLE I.

ALTERATION IN *p*H VALUES PRODUCED BY DIFFERENT SLAGS AFTER OVERNIGHT SHAKING.

Slag.	Clay loam 0·25% slag.	Sandy loam 0·5% slag.	Peat soil 1·5% slag.
14% "low-soluble"	6·00	4·89	5·2
16·5% "	5·88	4·96	5·2
11% "high-soluble" ..	6·22	5·42	5·5
14% "	6·36	5·58	—
16·5% "	6·46	5·68	5·6
Calcium carbonate	7·02	6·65	6·1
Blank	5·47	4·26	4·67

cyanamide, and five samples of basic slag, viz., 16.5 and 14 per cent. "low-soluble" and 11, 14 and 16.5 per cent. "high-soluble" slags.

A series of results obtained from the curves for the three soils with the different slags is submitted in Table 1.

It is interesting to observe how the results obtained for the sandy loam compare with those collected from a pot experiment which has been carried on for three years with the same soil (W), see fig. 7 p. 37. Different sets of pots were treated in the spring of 1931 with calcium hydroxide, 0.2 per cent. of which has raised the pH value of the soil from 4.33 to 5.31. Dressings of 0.2% of the 14 per cent. "high soluble" slag applied in spring 1933 raised the values from 4.33 to 4.91 and from 5.31 to 5.68: these figures are the averages of nine observations on quadruplicate pots during 1933. The curves, from which a few figures are given in Table 1, show that 0.3 per cent. of the same slag raises the pH values from 4.26 to 4.89 and from 5.30 to 5.72. Although this method, therefore, would seem to give fairly reasonable results with slag and is extremely useful in the estimation of "lime requirement" (7), appendix V. there is an obvious objection to it in an investigation of this kind.

Except in the case of calcium hydroxide or calcium carbonate, there are bound to be secondary effects, as a result of cation exchange, which depend upon the constitution of the fertiliser and the character of the soil. Furthermore, 18 hours contact is certainly not long enough to allow equilibrium to be reached with the less soluble substances.

To correct these errors, a series of experiments was carried out in which the suspension of water, soil and fertiliser was shaken at intervals daily by hand for one week. The suspension was then filtered and the soil washed with small portions of distilled/

Table II.

Alterations in *p*H Value Produced by Different Fertilizers after One Week Contact.

Fertilizer.	% on air dry soil.	Clay loam. Final suspension.	Clay loam. After washing.	Sandy loam. Final suspension.	Sandy loam. After washing.
None	—	5·56	6·29	4·35	4·80
Calcium carbonate ..	0·25	6·70	7·56	5·44	6·03
Superphosphate (14% or 16%)	1·25	4·98	5·54	3·87	4·43
Nitrochalk	0·76	6·64	7·64	5·50	6·44
Mineralphosphate 34%	2·50	6·00	6·63	5·20	5·72
" 26%	2·50	6·55	7·46	5·72	6·24
Calcium cyanamide ..	0·06	6·05	6·71	4·78	5·36
Basic slag 14% "low-soluble"	1·00	7·03	7·73	5·74	6·24
Basic slag 14% "high-soluble"	1·00	7·47	8·02	6·52	7·13

distilled water until the conductivity of the filtrate reached a uniform low value in each case. This treatment may be regarded as much more drastic than severe leaching of the soil by rain, but it gives a convenient basis for the comparison of results. The pH value of the soil was then compared with that of an original suspension before filtering.

The results obtained with two soils are presented in Table 2.

It will be observed that the longer contact and the washing have had an important influence upon the results. The quantities of material added to the soils are, of course, of quite a different order from those which would be used in practice, but they serve to show the different effects produced by different fertilisers. The two superphosphates increased the acidity to practically the same extent. All the other materials decreased the acidity. By preparing titration curves and interpolating at suitable points, it is possible to show that such dressings as are commonly employed in practice would have very small effects.

This was done in an experiment in which various amounts of the 14 per cent. superphosphate, of the 26 per cent. mineral phosphate and of two of the slags were shaken daily in soil suspensions over a period of three months.

Superphosphate. The pH value of the clay loam was not much affected up to additions of 0.5 per cent., being reduced from 6.33 to an average value of 6.19. Similar additions to the sandy loam had practically no effect, the average pH value being 5.01 as against 4.97 in the blank experiment. Additions of up to 1.5 per cent. had no significant effect on the peat soil.

Mineralphosphate. Successive additions to the clay loam up to 5 per cent. produced a regular slow increase in pH up to 7.7, so that/

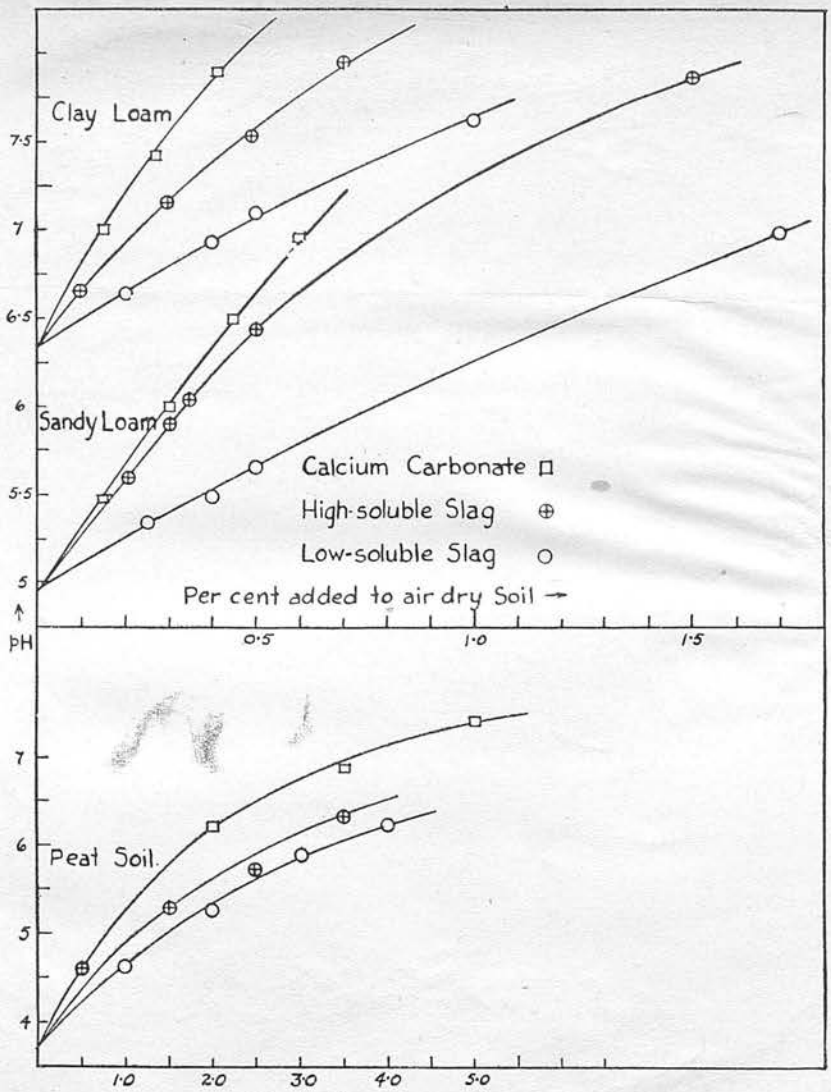

FIG. 1. TITRATION CURVES

TABLE III.

RELATIVE AMOUNTS OF CALCIUM CARBONATE AND TWO SLAGS REQUIRED TO PRODUCE SAME CHANGES IN SOIL ACIDITY.

Soil.	Treatment.	*p*H range.	Calcium carbonate.	14% "high-soluble."	14% "low-soluble."
Clay loam	3 months, washed ..	6·33–7·50	100	155	296
Sandy loam	"	4·97–7·00	100	117	262
Peat soil	"	3·69–6·00	100	147	188
Clay loam	Overnight, no washing	5·47–6·75	100	330	620
Sandy loam	" "	4·26–6·50	100	230	520
Peat soil	" "	4·67–5·70	100	180*	350

* 16·5 per cent. "high-soluble" slag. The peat soils were sampled at different times and are therefore not strictly comparable.

that an addition of 0.1 per cent. raised the pH value only from 6.33 to 6.40. With the sandy loam, a maximum addition of 4 per cent. raised the pH from 4.97 to 6.76, and a dressing equivalent to 0.1 per cent. to 5.10. In the case of the peat soil a maximum of 9 per cent. raised the value from 3.69 to 5.3, so that the ef effect of 0.1 per cent. is again less than 0.1 pH unit.

These results agree with those obtained in certain carefully conducted pot experiments (8) and field experiments extending over many years (4, 9) and from extensive observations in the field (6).

The curves for the basic slags are shown in fig. 1, and the results are summarised in Table 3 which also includes corresponding results from the first set of experiments in order to illustrate the importance of the time of reaction and washing of the soil.

It has been recognised for a long time (3) that slag contains a considerable amount of lime which is readily liberated and capable of acting as a base. It has also been shown that the lime content is comparable with that of calcium carbonate in fertility value (5) and in the reduction of the degree of unsaturation of the soil (10). The statement, however, that "1 cwt. of slag has about the same lime value as 1 cwt. of calcium carbonate" which appears in a recent bulletin published by the Ministry of Agriculture and Fisheries on Artificial Fertilisers (1) and is possibly based upon some preliminary observations in the Eighth Report of the Basic Slag Committee, is misleading and not supported by the results obtained in this investigation. Small dressings of carbonate or slag applied to an acid soil may show almost the same effect, but the titration curves shown here for the two slags and calcium carbonate are quite different for all three soils. Average values, calculated

from the curves, showing the relative amounts of the three materials required to bring about definite increases in the pH value of each soil are shown in Table 3. The ratios of the amounts of material for each soil were remarkably constant over the pH range selected. The prolonged contact has practically doubled the effect of the slags compared with the calcium carbonate.

In a laboratory experiment in which they added different materials to the soil and kept the mixture moist for several days, Brioux and Jouis (2) found that slag was approximately one third as effective as lime in decreasing the acidity of an acid sandy clay soil; in a similarly conducted experiment Williams (10) observed that, even on the basis of equal quantities of lime, a slag did not reduce soil acidity to the same extent as calcium carbonate or lime.

The neutralising value of the slag obviously depends upon the nature of the soil examined. It also depends upon the nature of the slag. The different effects produced by equal weights of different slags are illustrated by the figures of Table 1 and it seems that the "high-soluble" slags are much more effective than the "low-soluble" slags.

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APPENDIX V

The Estimation of the "Lime-requirement" of the Soil.

Information on the degree of unsaturation of a soil is extremely valuable since the figure is a well defined characteristic of the soil. But the determination of the degree of unsaturation is too laborious for the routine estimation of "lime-requirement" and is usually replaced by some simpler arbitrary determination which will give results accurate enough for advisory purposes. In assessing the "lime-requirement" of an acid soil, due attention is given to the cropping as well as to the soil properties, and the methods usually employed involve essentially the estimation of the absorption of a base under specific conditions. The results are closely dependent upon the experimental conditions, and the lack of complete agreement among different methods is, therefore, not surprising.

The displacement of hydrogen by calcium or any other base raises the pH value of the soil and the change depends upon the degree to which displacement has taken place. The extent of absorption and displacement, however, are related to the composition of the solution at the end of the reaction and are, therefore, not strictly comparable for different soils. The buffer capacity also varies considerably for different soils. Consequently, it would seem to be desirable to obtain more than one "lime-requirement" figure for any soil by whatever method the estimation is made. A comparatively short series of results for a soil showing the increase in pH value or the absorption of bases under slightly different conditions presents a much more complete picture of the properties and probable behaviour of the soil under field conditions.

The majority of soil samples which are received from the East of Scotland area for analysis are more or less acid, and the/

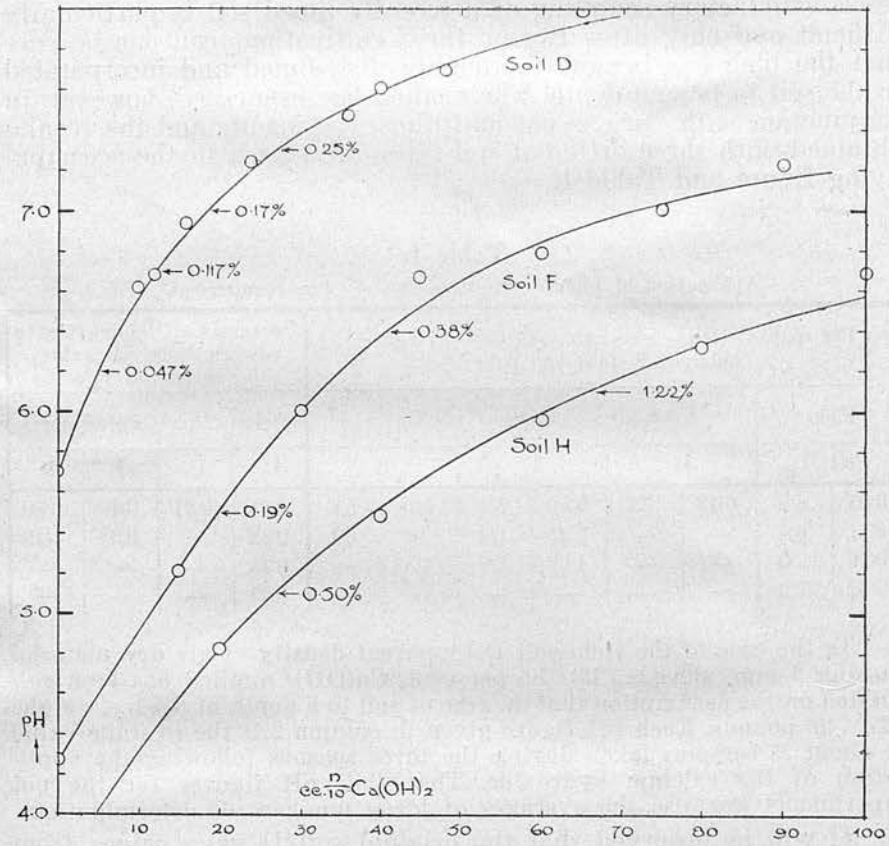

Fig. 1. Buffer Capacity Curves

the method for estimating their "lime-requirements" has been based on the above observations. It is a modification of the procedure recommended by Tovborg-Jensen (2) for estimating the buffer capacity of the soil and has already been described (1). The ratio for soil to n/10 $Ca(OH)_2$ solution is 1 : 2.5 except in the case of peaty soils when the ratio employed is 1 : 5 calculated to air dry soil. The n/10 calcium hydroxide solution can readily be obtained in presence of 2 per cent. sucrose. By this method, three or four points on the titration curve may be determined easily and quickly and are usually sufficient to give the necessary information for field practice: and 100 cc. of the undiluted solution is usually able to bring the pH values of the most acid and highly buffered mineral soils to about 7.

Sufficient data are not yet available to make a general statement on the relationship between laboratory and field figures, because satisfactory sampling of a recently limed soil is particularly difficult and, only after two or three cultivations, can one be sure that the lime has become thoroughly distributed and incorporated in the soil to plough depth. The method has been used, however, in conjunction with various pot and plot experiments, and the results obtained with three different soil types are shown in the accompanying figure and table 1.

Table 1.

Application of laboratory estimation of "lime-requirement".

(a) Per cent. $Ca(OH)_2$ added to soils and (b) pH values obtained in field and pots							Per cent. $Ca(OH)_2$ required to produce the pH values (b) in laboratory			
Field		Pots					Field	Pots		
Soil D		D		F		H	D	D	F	H
(a)	(b)	(a)	(b)	(a)	(b)	(a) (b)				
0.05	6.2	0.12	7.2	0.20	5.5	1.0 5.1	0.05	0.21	0.19	0.50
0.11	6.7	-	-	0.45	6.4	2.0 6.1	0.12	-	0.38	1.22
0.16	7.0	-	-	1.00	7.9	- -	0.17	-	-	-
0.32	7.3	-	-	-	-	- -	0.25	-	-	-

In the case of the field soil D (apparent density of air dry material passing a 2 mm. sieve = 1.15) the per cent. $Ca(OH)_2$ applied has been calculated on the assumption that an acre of soil to a depth of $8\frac{1}{2}$ ins. weighs 2.25×10^6 pounds. Each pH figure given in column 2 is the average value of about 25 samples taken during the three seasons following the application of the calcium hydroxide. The other pH figures for the pot experiments are also the averages of large numbers of determinations.

It will be observed that the original soil D gave values from the curve which approximate closely to those obtained in the field except in the case of the heaviest application of lime. During the fiest season, after the dressing of calcium hydroxide had been applied, the pH values of this plot were about 7.7; in the following seasons they occasionally fell below 7.0. For practical purposes, therefore, the method would seem to give a fairly true indication of which might be expected to take place under field conditions. As might be expected, the results from pot experiments differ inasmuch as the amount of calcium hydroxide calculated from the curve to bring about a certain change in acidity is much greater than what is actually required (21 : 12).

Soils F and H have been studied only in pots. They are abnormal soils, F being a very acid mineral soil and H an uncultivated acid peaty soil. In both cases the effects produced by the addition of calcium hydroxide to the pots are smaller than would be expected from the titration curves. For example, the pH value of F is raised to 6.4 by 0.45% $Ca(OH)_2$ in the pots and by 0.39% in the laboratory. The factor, however, is not constant. These two very acid soils, therefore, behave differently from the more normal soil D.

For routine purposes, the time of shaking the soil with calcium/

calcium hydroxide solution is about 18 hours - chosen entirely for convenience. Equilibrium is not always reached in that time but, apart from the higher points on the curves, the differences found by prolonging the time of shaking to 40 or 60 hours are usually not large.

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APPENDIX VI

The Determination of Nitrate in Soil Extracts.

There is an extensive literature dealing with the estimation of nitrate in soils and various workers have studied the question and written critically on different methods. On account of its simplicity, the phenol disulphonic acid method has naturally appealed to many soil investigators whose aim has been the observation of nitrification or the comparison of large numbers of samples rather than the accurate determination of absolute values. The main difficulty associated with this method is the interference of the yellow colour produced, by the organic matter in the soil extract, and the accuracy of the method depends upon the manner in which this organic matter is removed. Various means have been devised for this purpose and Harper (2) has made a useful review of the subject. The disconcertingly large number of papers criticising or recommending this or that precipitant furnishes sufficient warning against the adoption of any procedure without a preliminary examination under the conditions of any particular experiment.

The use of alumina as a precipitant of the organic matter has claimed a good deal of attention; but the comparatively recent suggestion of Skopintzew (4) to bring about the precipitation of the aluminium hydroxide at a definite pH value in the extract itself seemed to offer more adequate control of the reaction. It might be expected that the formation of the precipitant in situ, and under the best conditions for precipitation, would enhance its effectiveness and effect a general improvement in technique. Some preliminary experiments with this method demonstrated that the precipitate absorbed considerable amounts of nitrate and that a very much smaller quantity of the aluminium hydroxide than that recommended was sufficient to

produce/

produce perfectly clear solutions from the particular soil extracts under examination.

The phenol disulphonic acid was prepared by heating together 3g. phenol and 37g. sulphuric acid (sp. gr. 1.84) under an air condenser for 6 hours at 100°C. The reagent was stored in a coloured bottle.

For the precipitation of the flocculating agent, solutions of approximately 13 per cent. aluminium sulphate and 1.25 normal potassium hydroxide were prepared, and the relative amounts required, to produce a precipitate of aluminium hydroxide at pH 4.5, determined by means of bromcresol purple.

A standard solution of potassium nitrate was prepared from the recrystallised salt. A definite volume of this was evaporated to dryness in presence of 1 drop of alkali to prevent loss of nitrate, taken up with 2 c.c. sulphonic acid and diluted to a suitable point so that 1 c.c. was equivalent to 0.01 mgm. N. Any required amount of this solution was immediately ready for comparison purposes by simply making it alkaline with ammonia to develop the colour. The comparisons were carried out with a plunger type of Klett comparator, and it was found that concentrations of approximately 2 mgm. N/L were most suitable. In the soil extracts examined, the values lay between 0.3 and 16 mgm. N/L so that the amount of extract taken and the subsequent dilution of the coloured solution required careful attention.

In order to test the reliability of the technique at various stages, a series of potassium nitrate solutions, each containing 0.1 mgm. N, were examined as follows. In set 1, the solutions were evaporated to dryness, treated with sulphonic acid, diluted and made alkaline with ammonia. In set 2, they were passed through a filter, which was then washed three times, before the above procedure. In set 3, they each received 6 drops of the aluminium/

aluminium sulphate solution and the equivalent amount of potassium hydroxide, and, after being shaken and allowed to stand for a short time, were filtered and examined like those of set 2.

Complete recovery of nitrogen was obtained in the case of sets 1 and 2 but only 90 per cent. in the case of set 3. This indicated that no nitrate was lost in the process of filtering but that about 10 per cent. was lost in presence of the precipitant. It had previously been found that, for the soil extracts under examination, the above quantity of aluminium sulphate was sufficient to obtain a perfectly colourless solution, and that the amount recommended by Skopintzew, viz. 1 c.c., led to serious losses. Since the object of the investigation was to obtain relative, rather than absolute, values of nitrate in the same soil under different conditions, it was felt that a recovery approaching 90 per cent. was sufficiently accurate.

To test the technique more thoroughly, various soil extracts, with and without additions of nitrate, were examined, and the following example is typical of the results.

No.	Mgm. N in 50 c.c.			Percentage
	found	difference	added	recovery
1	0.09	-	-	-
2	0.133	0.043	0.05	86
3	0.166	0.076	0.10	76
4	0.197	0.107	0.15	78
5	0.250	0.160	0.20	80

The technique followed in obtaining the above results was finally adopted, and consisted in placing 25 or 50 c.c. of the fresh 1:5 soil:water extract in a boiling tube, adding 6 drops of aluminium sulphate and the requisite amount of potassium hydroxide, shaking, and allowing to stand overnight. The supernatant liquid was then poured through a filter and the precipitant washed thoroughly/

thoroughly three times under a strong jet of water. A drop of alkali was added to the filtrate, which was then evaporated to dryness; the basin was allowed to cool and 1 c.c. sulphonic acid was added and mixed with the residue. After standing for 15 minutes, the mixture was taken up with a little water, made alkaline with ammonia, and diluted to a suitable volume.

In the course of the last year or so, several papers have appeared dealing with the suitability of the xylenol method of determining the nitrate in soils (1, 3, 5), but the writer has not had an opportunity of testing it.

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The Effect of Nitrogenous Fertilisers upon the Protein Content of Oats.

INTRODUCTION.

In a series of investigations, started nearly twenty years ago in the United States, it was gradually established that, although the application of nitrogen at the early stages of growth produced the highest yields of wheat, the application at time of heading produced the best quality with regard to protein content (Davidson and Le Clerc, 6). Kraybill (17) confirmed these results and also showed how the composition, generally, was influenced by fertilisation and length of growing period. Gericke (13) has also demonstrated in pot experiments that the protein content of a spring wheat as well as of oats and rye could be markedly increased by delaying the application of nitrogen. The increase in protein content was further shown by Davidson and Shollenberger (7) to be associated with a wheat yielding as much flour as normal wheat, grown under the same conditions, and a bread of superior quality.

The economic importance, therefore, of a high protein wheat has been generally accepted in America, but emphasis has been laid by Mangels (19) on the influence exerted by climatic and soil factors and the difficulty of assessing protein content except by chemical analysis. In California, Alsberg and Griffing (1) have also demonstrated that climate is a more important factor than either variety or soil in determining the quality of wheat, for the ratio of protein to starch depends largely upon moisture conditions. In other words, the texture of the soil exerts a greater influence than its chemical properties, and that is confirmed by results reported from Canada by Shutt and Hamilton (26), who found that the best grain was produced on soils which dried out in the ripening stage. Opitz (22), however, maintains that, although there is a general climatic effect upon the various properties of cereal grains in Germany, nitrogenous fertilisers do exert/

exert an influence upon their quality and chemical and physical properties.

In this country, on the other hand, Fisher and Jones (10) found no correlation between protein content and baking quality and no significant increase in protein content due to manurial treatment; whilst Forster and Vasey (11) assert that, under the growing conditions in Victoria, Australia, nitrogenous top dressings for wheat do not find favour on account of the non-drought resistant type of growth produced.

These observations are sufficient to indicate that, although the composition of a crop like wheat may be artificially influenced to some extent, the effects of such factors as climate, soil and variety make the problem a most complex one.

In the case of barley, the position is different, because the valuation for malting purposes is lowered by a high nitrogen content of the grain. The aim in the growing of barley is, therefore, to obtain a satisfactory yield without increasing the nitrogen in the dry matter, and in a study of this problem, extending over ten years, Russell and Bishop (25) have shown how such factors as seasonal rainfall and temperature, soil type, manuring and cultural operations all play a part. They demonstrated that soil and season have pronounced and equal effects and that a late application of nitrogen intensified the effect of over manuring with nitrogen in increasing the protein content of the grain. Similar results are reported by Bennett (4).

Unlike barley, an increase in the protein content of oats would enhance its value, for it is used almost exclusively for feeding purposes, and home produced foodstuffs alone must be supplemented by imported protein-rich material. Although a fair amount of work has been carried out on the composition and metabolism of the oat plant, very little attention has been given to what/

what seems to be the only feasible method of controlling the nitrogen content of the grain, namely, the time of application of nitrogen to the growing crop.

In an early paper, Hendrick and Greig (14) showed that, for the North of Scotland, the protein content was lower in dull, cool seasons than in dry seasons, whilst Berry (3), in an exhaustive study of the subject, came to the conclusion that the oat crop was more subject to variation than other cereals on account of the wide range of conditions under which it might be grown. The characteristics most subject to variation were nitrogen content and kernel weight, and he showed that there was a large variation within any one variety from year to year and, also, that, in any one year, locality might exert an influence far in excess of that due to moderate manuring and time of sowing. He observed that a long or, especially, a short growing period produced grain having a nitrogen content above the average, due respectively to immature crop and premature ripening. Fagan (8) and Wagner (30) have studied the variation in nitrogen content during growth, whilst Stahl and Shive (28) have examined the rate of nitrogen absorption by the plant in culture solutions. Generally speaking, the absorption of nitrogen never ceases entirely but its rate reaches maxima in the early stage and again during flowering, which correspond to breaks in the gradual decline in the percentage of nitrogen in the plant between germination and ripening: the proportion of nitrogen also increases in the spikelet during ripening and decreases in the straw between the beginning of panicle formation and the "milky ripe" stage. With minor exceptions, these data fall into line with Berry's views that the absorption of nitrogen by the plant is practically complete at the beginning of July and that the storage of nitrogen in the kernel is derived entirely from the supplies in the straw, husk/

husk and chaff which lose nitrogen between June and harvest.

A number of papers have also appeared dealing with the effect of nitrogenous fertilisers on the growth and composition of the oat plant. Blanck, Giesecke and Heukeshoven (5) have shown, in sand culture experiments, that, with increasing nitrogen supply, the period of maximum dry matter production in the aerial parts is shortened; whilst Tornau and Meyer (29) have shown that the growth period and maximum yield are increased with increasing supplies of nitrogen and that the utilisation of nitrogen increases with the content of moisture in the soil. Pfützer (23) and Kruger (18) have also demonstrated that the intake of nitrogen is influenced largely by the amount of soil moisture. Mix (20) has also obtained results which show that, although the nitrogen content of oats is partly inherited and undoubtedly affected by climate, soil and manuring, it is usually increased by late sowing and rising temperature up to tillering, and decreased by high rainfall after tillering. These observations are all more or less what would have been expected from the earlier work mentioned, and serve to illustrate the variety of factors which is likely to influence the final protein content of the grain. There seems to be only one paper, however, which deals with the possibilities of increasing the nitrogen content of the grain by delaying the application of nitrogen.

In 1922, Gericke (12) reported the results which he obtained in a set of greenhouse pot culture experiments with a soil deficient in nitrogen. The conditions were undoubtedly suitable for obtaining definite responses, because there were no disturbing effects due to climate, including soil moisture, and locality, and the amount of nitrogen applied, equivalent to about 100 lb. per acre, was far in excess of what would be permissible under normal field conditions. The chief observations may be summarised/

summarised as follows :- The tallest plants were produced in the series of early dressings, and nitrogen, applied to progressively older plants, produced correspondingly shorter stalks but a larger number of them so that, actually up to a certain age of the plants, the later the application of nitrogen the greater the amount of dry matter produced. Although the first plants to reach maturity were those receiving the earlier dressings of nitrogen, the length of the growing period of the head-bearing stalks was decreased as the application of nitrogen was delayed. That was because tillering did not commence until nitrogen was applied and, the later the tillers started, the shorter their period of growth. The untreated cultures were the last to mature. Finally, there was a progressive increase in the protein content of the grain as the time of application of nitrogen was delayed. Actually, there was more than twice as much protein in the grain from the cultures receiving the latest treatment as in the grain from those which received nitrogen at the time of planting. Gericke himself has emphasised the fact that such conclusive results would probably not be obtained from other soils, but his figures seem to remove any doubt about the existence of physiological differences of oat plants at different stages of growth and the manner in which nitrogen affects the quantity and quality of the product.

In spite of the importance of climate and locality in affecting the composition of the grain, there appeared to be considerable grounds for believing, therefore, that the result of delaying the application of nitrogen in the field would be a tendency to increase the protein content of the grain. It was, of course, realised that experiments would have to be continued over several seasons in order to collect conclusive evidence, and that it would be necessary to confine comparisons to the actual differences/

differences obtained in different years. In other words, on account of variations in any variety due to climate and locality, it was unlikely that any study, beyond that of the effect of time of application of nitrogen on protein content from year to year, would yield satisfactory results within a reasonable time. In the experiments to be described, therefore, no attempt was made to study the same variety each year, and the soils were not selected for their responses to nitrogen but were accepted as normal arable soils under rotation, none of which could be described as abnormally deficient in nitrogen. The primary object of the investigation was to determine whether the protein content of the grain could be increased under ordinary farming conditions without prejudice to harvest operations, and the monetary value of such a change.

TECHNIQUE.

The area selected for experiment was usually simply a section of a field, removed from headland influences, which was sown in the usual manner. Strips about one foot wide, separating the plots, were kept hoed during the season and the crop was cut by scythe. The produce from each plot was tied into sheaves and, after a suitable lapse of time, determined by weather conditions, the grain was stripped from the sheaves and threshed by hand. Some time usually elapsed before the analyses could be undertaken, but all the samples from an experiment were analysed in the course of a few days. In the larger plot field trials, samples of the grain were usually collected immediately before cutting commenced.

The number of grains in a carefully sampled and weighed position were counted to obtain an estimate of the weight of 100 grains.

A large sample of the produce from each plot was ground in a Christie and Norris mill, and duplicate sub-samples were dried overnight/

overnight at 100-105°C. to get the moisture content of the grain. The oven dried samples were than examined for nitrogen by the usual Kjeldahl method. The moisture and nitrogen were calculated in terms of airdry and ovendry material respectively. Representative samples of the grain were also valued each year by one or more seedsmen.

For convenience in making comparisons, the analytical results are given both as percentages and as relative values with the means equal to 100.

1929 Experiment.

Locality. Horse Park, Boghall.

Variety. Castleton, after a clover hay mixture.

Experiment. 6 plots, each 8 sq.yd.; duplicates treated with $(NH_4)_2SO_4$ in solution at the rate of 64 lb. N/A on the following dates :-

Plot 1. 6th April; a few days after sowing.

" 2. 9th May; plants 3 inches high.

" 3. 7th June; plants 6-8 inches high: no noticeable differences between plots.

Date of cutting - 20th September.

Weather. The second halves of May, June and July were very dry with some fairly high temperatures in July. The first half of August was cool and wet and a prolonged spell of warm dry weather did not come until September.

Valuation of grain. Estimates of the relative values of representative samples from the six plots, by different seedsmen, were not in agreement; all the samples were assessed as of good milling quality and valued at about 21/- ± 3d. per quarter.

Results. Plots	1	2	3
Wt. in g./100 grains	3.02, 3.12	3.02, 3.03	3.07, 3.09
Per cent. moisture	10.75, 10.58	10.86, 10.73	10.68, 10.54
Per cent. N.	1.64, 1.61	1.59, 1.75	1.62, 1.72

Conclusions. The differences, due to treatment, in grain weight and per cent. nitrogen are sometimes less than the variation in duplicate plots, and a critical analysis of the data is valueless. There is a suggestion that the moisture content is lowest in the case of the grain from plots 3, but the number of results is insufficient for any precise statement to that effect.

This preliminary experiment indicated that (a) the rate of application was too small or (b) the time intervals between applications were too short or (c) the soil was relatively rich in available N after the clover or (d) the season was such as to repress the effects of the dressings. The dressing of nitrogen was already more than could reasonably be recommended in practice, and it was felt that the lack of response might be attributed to the shortness of the plants at the beginning of June and the prolonged period of growth.

1930 Experiment.

Locality. March Park, Boghall.

Variety. Sandy oats.

Experiment. A 4 × 4 Latin Square, each plot 4 sq.yd.; treatments

60 lb. N per acre as $NaNO_3$ applied in solution as follows:-

Plots A. 28th April, plants 3" high.

Plots B. 20th May, " 5" . " . "A" plots markedly advanced.

Plots C. 10th June, "A" plots (12" high) and "B" advanced compared with plots C and D. On the 30th June Plots D were very pale in colour and lagging far behind.

Plots D. No treatment.

Date of cutting - 8th September.

Weather. The second half of May and the first halves of June and July were very dry with some fairly high temperatures: the second half of August had two periods of high rainfall and temperature, while the beginning of September was dry and warm.

Valuation/

Valuation of grain. All the samples from different treatments were assessed as of first class milling quality or second class seed and valued at 17/6d. per quarter.

Results.

Plots	A	B	C	D	Mean	Standard Error.
Weight in g. of 100 grains	3.75 97.1	3.85 99.8	4.01 103.9	3.85 99.2	3.86 100.0	0.042 1.08
Percentage Moisture	10.86 106.1	9.98 97.6	9.61 93.9	10.47 102.4	10.23 100.0	0.189 1.84
Percentage Nitrogen	1.87 98.4	1.99 104.4	2.00 105.1	1.79 93.9	1.91 100.0	0.029 1.50

Conclusions. In each of the three determinations the effect of treatment is undoubtedly significant. The analyses of variances are given on p.125 and an example of the complete calculation for a Latin Square experiment on p.126 . The significance of the differences between treatments has been examined by the z test. It will be seen that, in the case of both grain weight and moisture, the ratios of the mean square for treatments to the mean square for errors would be exceeded by chance in something less than 5 per cent. of trials. In the case of nitrogen, chance would allow z to exceed 1.14 in 1 per cent. of trials and the observed value is 1.38 and clearly significant.

It is now possible to compare the effects of the different treatments, differences between the results greater than about three times the standard error being regarded as quite significant.

(a) Grain weight. The average grain weight from C plots, receiving the latest dressing, is significantly above that of all other plots; and that from B plots, receiving the second dressing, might be regarded as significantly greater than that from plots A receiving the early dressing.

(b) Moisture/

(b) Moisture. The moisture content of the grain from plots C is significantly less than that from plots A and D; and the grain from plots B has a moisture content significantly less than that from plot A.

(c) Nitrogen. The early application of nitrogen, plots A, has effected a significant increase in the nitrogen content of the grain over the untreated plots D; the later dressings, plots C and B, have effected a still further increase over plots A.

By way of summing up the evidence from this experiment, it may be said with confidence that a dressing of nitrogen, in the form of sodium nitrate, at the rate of 60 lb. N per acre, has increased the nitrogen content of the grain and that this increase is greater when the dressing is delayed from the end of April to the beginning of June. The later the time of application of the nitrogen up to the 10th June the greater the weight of the individual grains and the lower the moisture content of the grain.

These results were regarded as being of considerable practical importance, but since they might have been influenced by variety or soil or season and since the dressings of nitrogen were probably greater than the average farmer would dare apply in practice, it was decided to carry the investigation a stage further. It was desirable (a) to confirm the effect of 60 lb. N as sodium nitrate applied when the plant was more than 6 inches high, (b) to test the relative effeciency of ammonium sulphate and sodium nitrate, (c) to examine the influence of a smaller dressing, (d) to observe the effect on ripening and crop yield.

1931 Experiment.

Locality. Dolphingstone.

Variety. Abundance.

Experiment. A 5 × 5 Latin Square, each plot 4 sq.yd., dressings

of/

of nitrogen applied in solution, the untreated plots receiving water only.

Plots A, 19th May ; plants 4" high; 30 lb. N/A as $(NH_4)_2SO_4$

" B, " ; " " " ; " " " $NaNO_3$

" C, 16th June; " 14-18" " ; " " " $(NH_4)_2SO_4$

" D, " ; " " " ; " " " $NaNO_3$

" E, " ; " " " ; 60 lb. N/A " $NaNO_3$

Date of cutting, 5th October.

Weather. The summer of 1931 was abnormally wet, there being very heavy rains during the first half of June and throughout August. The result was that nearly all the plots were badly twisted and laid and were still rather green at the beginning of September. The adjoining area, which had received no nitrogen, seemed to ripen rather sooner than the plots, but it had also lodged badly. Although the grain was ultimately obtained in fairly good condition, the idea of weighing the crop had to be abandoned.

Valuation of grain. No appreciable difference could be discerned between representative samples, which were all classed as of good average milling quality.

Results. A complete statement of the figures for nitrogen together with the statistical analysis of the results are given on pp.126. Only a brief summary of all the results is given below.

Plots	A	B	C	D	E	Mean	Standard Error
Weight in g. of	4.12	3.79	3.85	3.81	3.68	3.85	0.09
100 grains	106.8	98.4	99.8	99.0	95.5	100.0	2.35
Percentage	12.75	12.52	12.62	12.53	12.70	12.62	0.12
Moisture	100.9	99.2	100.0	99.3	100.7	100.0	0.91
Percentage	2.27	2.28	2.33	2.31	2.52	2.34	0.04
Nitrogen	96.9	97.2	99.5	98.5	107.7	100.0	1.82

Conclusions. In such an experiment, with 4 degrees of freedom for/

for treatments and 12 for errors, chance will allow the ratio z to exceed 0.844 in 1 per cent. of trials and 0.591 in 5 per cent. of trials. In the case of grain weight, the observed value for z is 0.574 so that the effect of treatment is barely significant. At the same time it is noteworthy that the value for plots A exceeds the values for each of the other plots by three times the standard error, and the test may be regarded as valid for the contrasts which the experiment was designed to test. It may be said, therefore, that the early dressing of ammonium sulphate produced a heavier grain than either the late dressing or the sodium nitrate.

There has been no response to different treatments in the case of moisture, the mean square for treatments being actually smaller than that for errors. In spite of the low standard error, the different values do not depart materially from the general mean.

In the case of nitrogen, the observed z value is 0.901 which would be exceeded by chance less than once in a hundred trials and is therefore undoubtedly significant. Apart from plot E, however, the average values do not differ materially.

To sum up the results of the experiment, therefore, it could be stated that the early dressing of ammonium sulphate produced the largest grain weight while the late double dressing of sodium nitrate effected an increase in the nitrogen content of the grain. The results were not nearly so definite and important as those obtained in the 1930 experiment and the lack of agreement between the two sets of data was rather disconcerting. The lack of response to the late smaller dressings of nitrogen could in part be attributed to the fact that even the early dressings showed no response in the percentage of nitrogen in the grain. This may be related to the abnormally high percentage of nitrogen in/

in the grain. Although the comparison is not quite valid, it might be mentioned that a representative sample of grain taken from the untreated area surrounding the plots contained practically the same percentage of nitrogen, viz. 2.28, as plots A and B: for the same sample, the weight of 100 grains was 4.14, again similar to the average value for plots A, but the moisture content of the grain was only 12.23 which was very much less than the mean for all plots. As previously mentioned, the season was an abnormally bad one for grain crops, and the twisted and lodged condition of the crop towards the end of summer was not conducive to a satisfactory completion of the experiment. In surveying the results, therefore, there was a tendency to lay emphasis upon the seasonal factor and the highly fertile condition of the soil. No definite conclusions regarding the susceptibility of the crop to lodge under different treatments could be arrived at for the simple reason that in the summer 1931, nearly all crops suffered severely, but in view of the feeling prevalent in many farming areas that a late dressing of nitrogen would naturally retard ripening and increase any tendency to lodging, it was decided to carry out a simple experiment on a larger scale in 1932.

1932 Trial.

Locality. Boghall Experimental Farm.

Variety. Yielder.

Experiment. This consisted of four $\frac{1}{4}$ acre plots on which fertiliser was broadcast as follows :-

Plot 1. 7th June; plants 6-9" high; 23 lb. N/A as $(NH_4)_2SO_4$

" 2. --------; " " " ; no treatment.

" 3. 23 lb. N/A as $(NH_4)_2SO_4$ + 2 cwt. super phosphate/A at seeding

" 4. " " " " " " " " " " "

Date of cutting, 29th August.

Weather. The summer was generally dry and, at time of cutting, the/

the crop was barely so ripe on plot 1 as on the others. This might have been due to the fact that, although the nitrogen was applied on 7th June, there was no rain until three weeks later so that the nitrogen could scarcely have exerted any influence before the end of June. July was warm with the rainfall well distributed, while the middle of August was dry and warm. There was little apparent difference in the length of straw which, generally speaking, was short.

Valuation of grain. All four samples were regarded as of good milling quality and valued at 18/- per quarter by seed merchants.

Results.

Plot	1	2	3	4
Weight in g. of 100 grains	4.07	3.92	4.17	3.98
Per cent. moisture	13.27	12.81	12.76	12.21
Per cent. nitrogen	1.89	1.67	1.66	1.60

Conclusions. It is, of course, impossible to stress any differences which exist between the above values for different plots for there is no available estimate of normal variation over the field. The experiment, in fact, was not intended to be more than a qualitative test of the effect of a late dressing of nitrogen on maturity and extent of lodging, and the season was such that the information desired was not forthcoming. However, if account is taken of the fact that plots 3 and 4 were duplicate plots, not adjoining each other, some idea of the range of variability is available. With earlier values for standard error in mind, it might then be said that, in all probability, the late dressing of nitrogen has increased the nitrogen content of the grain. The increase is actually of the order of 10-15 per cent., but naturally cannot be stated precisely.

It was obvious that the question merited still further investigation/

investigation and in 1933 it was decided to continue with dressings of about 1 cwt. ammonium sulphate per acre and contrast a late dressing with an early dressing and with no dressing. An attempt to obtain an estimate of yield was also decided upon in addition to observations on time of ripening and susceptibility to lodging.

1933 Experiment.

Locality. Boghall.

Variety. Victory.

Experiment. The experiment was carried out on two sets, A and B, of randomised blocks, each set having previously consisted of five large plots which had been variously treated since 1929 with sulphur or calcium hydroxide in a study of the effect of soil acidity upon various crops. Details of these treatments will be found on p.27 . Each of these large plots was subdivided into three equal plots of 8 sq.yd. to give 5 blocks of three plots in each set. The treatments (1) nil,(2) dressing at time of sowing,(3) late dressing, were randomised in each block. Hence there were three treatments repeated five times in each set. On account of the artificial variation in soil acidity, the blocks were not exact replicates, but, as will be shown later, the effects due to acidity were negligible. The nitrogen was carefully distributed by hand in the form of ammonium sulphate.

Set A, 19th April; oats sown; plots 2 received 40 lb N/A.

" , 7th June ; plants 12-18" high; plots 3 received 40 lb N/A

Set B, 17th April; oats sown; plots 2 " " "

" 7th June ; plants 12-18" high; plots 3 " " "

Weather and General Remarks. On the 7th June, after a period of moist weather with steadily rising temperatures, plots 2 were markedly advanced both in bulk and colour. At the end of June plots 1 were yellowish and short compared with 2 and 3, and/

and the plants were much taller on plots 2 than on plots 3. This same state of affairs still held good at the middle of July, after periods of hot weather, when it was further observed that Set A generally was yielding a better crop than set B. That was expected to a certain extent since, in 1932, the plots of set A yielded a good crop of beans while those on set B were fallowed. Heavy rains towards the end of July were succeeded by a fairly dry, warm spell, and at the beginning of August the crop in set B was standing well and ripening fairly evenly while the crop in set A was somewhat twisted and laid in places, usually, but not invariably, on plots 2 and 3. The crop was cut on the 23rd August and it was definitely riper on set B than on set A, but not so heavy; as revealed by the following table on plot yield, the crop was much lighter from plots 1 than from plots 2 and 3.

Valuation of grain. Representative samples of the grain from all the plots 1, 2 and 3 in each set were submitted as usual for valuation by seedsmen and they were valued at 16/- per quarter.

Results.

		Treatment				
		1	2	3	Mean	S.E.
Set A	Yield in lb.	12.4	13.9	14.6	13.63	0.72
	Grain wt. in g/100	3.23	3.03	3.06	3.11	0.08
	% moisture	11.55	11.61	11.70	11.62	-
	% nitrogen	1.89	1.99	2.05	1.98	0.02
	" (rel.)	95.5	100.5	103.5	100.0	1.08
Set B	Yield in lb.	9.4	10.7	12.2	10.73	0.45
	Grain wt. in g/100	3.14	3.14	3.30	3.19	0.06
	% moisture	11.79	11.79	11.68	11.76	-
	% nitrogen	1.86	1.85	1.97	1.90	0.01
	" (rel.)	98.4	97.9	104.3	100.0	0.99

Conclusions. (a) Yield. Throughout the season, plot 3, in block 5 of set B, was much inferior to all the others, due, it was felt, partly to unsuccessful seeding and partly to the activity of mice. The actual yield of straw and grain from the plot was only 8 lb. and/

and obviously so abnormal that it was substituted by the figure 13.56, calculated from the remaining 14 values in the set as shown on p. 129. The experimental error involved in harvesting and weighing the produce from such small plots was necessarily large, but in both sets the treatment with nitrogen has effected an increase in crop yield, and, in set B, the late dressing has given a greater yield than the early dressing. The relative yields of straw and grain were not measured.

(b) Grain weight. The effects of nitrogen, either as an early or as a late dressing, on the grain weight are negligible and the same may be said of (c) moisture content.

(d) Nitrogen in the grain. The effects of the applications of nitrogen on the nitrogen content of the grain are quite clear. The early dressing has brought about an increase in set A but not in set B; the late dressing has increased the nitrogen content over the early dressing in both sets. The lack of response to the early dressing in set B is rather surprising in view of the observations made during the growing season and mentioned above. It was fully expected that the responses to both early and late dressings would be more marked in B than in A. The darker colour of the crop in plots 2 in June was evidently misleading for, although it eventually led to an increase in percentage nitrogen but no definite increase in yield in set A, it meant an increase in yield but no increase in percentage nitrogen in set B. The ultimate effect, namely an absolute gain in nitrogen, was, of course, the same in both cases and only what might have been expected under ordinary conditions. There was undoubtedly a greater response to the late dressing in set B than in set A, and that is possibly related to the fact that the percentage of nitrogen in the grain was lower throughout in set B. The increase in crop yield, in addition to the increase in nitrogen content of the/

the grain, as a result of the late dressing, is of considerable importance since it is generally recognised that an early dressing tends to increase the ratio of straw to grain. It establishes, for the particular conditions of the experiment, the value of delaying the application of nitrogenous fertilisers until the crop is about knee high.

It is desirable at this point to deal with the influence of the variation in soil acidity on these results. The average pH figures for the blocks during the period April to August 1933 were as follows :-

Block	1	2	3	4	5
Set A	4.87	5.28	5.72	6.19	7.30
Set B	5.06	5.48	5.95	6.13	6.65

The complete sets of experimental data are given on pp. 129 in the statistical treatment of the results. It will be observed that in every case the block "mean square" and error "mean square" are substantially the same, indicating that there are no real "block" differences; that is to say, the effects of differences in soil acidity are within the limits of sampling error. In this respect the results differ from those quoted by Hansen (15) and Nehring (21a). The latter obtained a 10 per cent. increase in yield and an 8-15 per cent. increase in nitrogen content by raising the pH of an acid soil from 5.2 to 7.9 with additions of lime. Mix (20), on the other hand, submits evidence that lime increases the nitrogen content of the grain but reduces the yield. Nehring also found that the moisture content of the grain increased with increase in the pH value of the soil. It is extremely probable, however, that greater responses to liming would in general be obtained with such an acid, humus-poor, mineral soil as he used in his pot experiments than with the well buffered soil concerned in the above experiment: cf. section I p.21.

It/

It has been shown by Smith and Robertson (27) that the plot yields for crops of barley, potatoes and clover hay on these particular sets of plots in preceding years did not vary much from the mean; but that with an acid infertile soil the responses in yield and composition of crops to liming were very large. In a later paper, Nehring states (21b) that the nitrogen intake of oats in various acid soils was only slightly influenced by pH. As a result of a large number of field experiments in Sweden, Åslander (2) concluded that, even with barley, the yield depended more upon soil conditions, cultivation, etc. than upon acidity, if the pH was somewhere between 5 and 8. The literature on the subject of soil acidity and crop yield is very extensice and, judging by the diversity of opinion expressed, it is obvious that acidity alone is usually of secondary importance and that the general character of the soil, the crop and the season play a much more important part. Roberts (24), for example, has drawn attention to the large seasonal variations in crop yield in Wales, which cannot be correlated with total rainfall or accumulated temperature and demonstrated that it is a very complex problem.

The above results, taken in conjunction with those of the previous four years, seemed to dispel any doubt that a valuable increase in the protein content of oats could be secured under favourable conditions with only a minor alteration in farm practice. There still remained some doubt, however, regarding the effect on harvest operations, for the field experiment designed to test this point in 1932 was inconclusive as a result of the particularly early harvest. It was decided, therefore, to lay down trials in 1934 on a field scale at different localities with the primary object of securing further information on the effects of delaying the application of nitrogen on the ripening, susceptibility to lodging and general conditions at time of cutting./

cutting. It was also felt that the places chosen should be in "late" rather than "early" districts so that the test should be as severe as possible.

1934 Trials.

Trials were arranged in four places and the experimental details and observations are given below.

Place	Boghall	Cocklaw	Baads Mains	Crookston
Elevation	600 ft.	350 ft.	650 ft.	780 ft.
Previous crop	Grass	Roots	Grassland	Potatoes
Variety sown	Yielder	Victory	Yielder	Yielder
Dressing in lb N/A	23	17	23	23
Late dressing on	31st May	25th June	25th June	26th June
Time of cutting	23rd Aug.	16th Aug.	30th Aug.	27th Aug.

The fields were sown as usual and strips of $\frac{1}{4}$ to $\frac{1}{2}$ acre in size were marked off. In each case the nitrogen was applied in the form of ammonium sulphate.

At Boghall (a), two strips received nitrogen at the time of sowing (29th March) in addition to 2 cwt. per acre of superphosphate; other two strips received the same amount of superphosphate at seeding time and the nitrogen two months later. As the growing season advanced, it looked as if the early dressing was producing taller stems and this was confirmed when the crops were cut. At the end of July, the general opinion was that the crop which received the early dressing was also ripening rather sooner but, at the time of cutting, the difference was not noteworthy. There was a little lodging but that was confined to the areas which received the early dressing, probably on account of the longer straw. Samples were taken immediately after cutting from each of the four strips.

At Cocklaw (near Currie) (b), one strip received a dressing of mixed fertiliser (containing 2 cwt. superphosphate, 1 cwt. ammonium sulphate and 1 cwt. 30 per cent. potash salts) at the/

the rate of 3 cwt. per acre. To a second strip the nitrogen was not applied until the end of June. On the 5th June the crop was thin and no response to the early dressing was noticeable. When harvest commenced, the area which received the late dressing was not quite so ripe as the other but the difference was negligible and did not affect the time of cutting. There was no lodging. Two independent samples were taken from each strip just before cutting.

At Baads Mains (near West Calder) (c) the whole field received 2 cwt. superphosphate and 1 cwt. 30 per cent. potash salts per acre before sowing and all except one strip received 1 cwt. ammonium sulphate per acre at time of brairding (11th May). One half of the strip received no nitrogen, the other half a late dressing. On account of heavy rains, large areas of the crop were laid quite irrespective of the treatments, although it seemed as if the area which received no nitrogen had suffered least. The ripening was also patchy and no definite difference could be ascribed to the time of applying the nitrogen. Samples were taken from the areas of "no", "early" and "late" applications of nitrogen the day before it was proposed to cut.

At Crookston (d), the field, except on two strips, received 1 cwt. ammonium sulphate at sowing time. One strip received the same dressing when the corn was about 18" high; the other strip received no nitrogen. In the early part of the season, the crop on these strips was noticeably lagging behind that on the rest of the field and at cutting time the straw on the "early" nitrogen parts was definitely the tallest. There were,however, no apparent differences in the degree of ripeness as a result of the different treatments. Certain areas had lodged badly, but they occurred quite irregularly and bore no relation to treatment. The grain on the "no", "early" and "late" nitrogen areas was sampled just before cutting.

Weather and Observations. Season 1934 was again an "early" one for harvest, although not so "early" as 1933 on account of the rather unsettled weather after the beginning of August. On the lower ground the straw was generally short on account of the dry spell in June and July but on the higher ground, and particularly at the places where the above trials were conducted, the straw was quite normal in length so that the heavy rains in August provided a good test of the liability of the crops to lodge as a result of a late dressing of nitrogen. It can confidently be stated that where lodging took place it was quite independent of the time of applying the nitrogen or occurred on the areas which received an early dressing and which usually carried a longer straw. The late dressing did undoubtedly show a tendency to delay the ripening but at none of the places concerned was it considered by the farmers to be of any consequence.

Results. The results obtained in the analyses of the samples of grain are summarised below.

Effect of time of application of nitrogen on properties of grain.

Nitrogen Applied.		Boghall	Cocklaw	Baads Mains	Crookston
None	Grain weight	-	-	4.01	3.85
	% Moisture	-	-	14.29	13.48
	% Nitrogen	-	-	1.86	1.73
Early	Grain weight	4.19	3.93	3.87	3.90
	% Moisture	14.62	14.64	14.29	13.78
	% Nitrogen	(a) 1.76	1.66	1.88	1.66
		(b) 1.83	1.65		
Late	Grain weight	4.30	3.35	4.32	3.65
	% Moisture	14.59	14.55	14.08	13.92
	% Nitrogen	(a) 1.85	1.88	1.95	1.75
		(b) 1.85	1.98		

Conclusions. The nature of the experiments preclude any statement on the precise response to treatment. In these cases where duplicate field samples were taken the differences between duplicates were less than the differences due to treatment and it is permissible to draw some general conclusions. The figures/

figures for grain weight and moisture content are again quite irregular, but the figures for nitrogen tend to confirm previous observations. In each case the nitrogen content of the grain is increased by delaying the application of fertiliser. In the case of Cocklaw, the very large increase is similar to that obtained at Boghall in 1932 and may be associated with the relatively light crop and low percentage of nitrogen in the grain. In the other three cases, the increases are of the same order as those previously obtained.

General Discussion of Results.

In the last five of the six years' experiments, there has been fairly conclusive evidence that a late application of nitrogen to the crop produces an important change in the composition of the grain. The first experiment was essentially a preliminary trial and the results do not carry so much weight as the others. There seems to be some justification for assuming, therefore, that it is quite possible under field conditions to increase the amount of nitrogen in the grain of oats and so increase its feeding value. The economic and practical aspects of the question remain to be examined.

(a) The effect upon the other constituents of the grain. Since the chief constituent of the grain consists of carbohydrates, a small percentage difference due to an increase in crude protein is of little or no account. The "ether extract", however, is of considerable importance in the feeding value of oats and a sensible reduction in this would offset the advantage of an increase in protein. No determinations of the fat were made but Mix (20) has shown that there is no relationship between the nitrogen and oil in oats. With respect to grain weight, the effects of nitrogen are not consistent. In 1930, nitrogen increased the grain weight and the increase was greater the later the application of fertiliser. In the other five years the results

were quite indefinite. Berry (3) showed that there was no connection between the size of the kernel and its nitrogen content and, in the case of barley, Russell and Bishop (25) produced evidence that there was no response in corn weight to early or late nitrogenous dressing. Gericke also found (13) that the size of the grains of wheat, oats and rye, as indicated by the weight of 100 kernels, was not markedly affected by the addition of nitrogen at various stages of plant growth. The market value of grain is undoubtedly related to "bushel weight", but there seems to be no reason to believe that it would be affected by the treatment required to increase the percentage of protein, and when the grain is intended for feeding purposes "bushel weight" becomes of secondary importance.

(b) The effect upon ripening and lodging. In the earlier plot experiments, no definite conclusions could be formed on the influence of the fairly large dressings of nitrogen on the ripening of the grain, and any lodging that occurred did not seem to be related to treatment. In the last three years, ripening did seem to be delayed somewhat by the late dressing, but the harvests were early and the effect was regarded as of little importance with an oat crop. In 1932, the straw was short and there was no lodging. In 1933, the straw was fairly long and a certain amount of lodging took place but on quite unrelated areas. In 1934, the trials were designed principally to test this question more thoroughly and the results have already been fully discussed.

(c) The effect on yield. With respect to this question, figures were given in the 1933 experiment which showed that the delayed application of nitrogen increased the total yield of straw and grain. Reliable conclusions could not be based on the results for one year and figures for the 1934 trials will not be available until/

until the crops are thrashed. The point is an important one because there could be little justification in attempting to increase the protein content if the yield of grain were markedly reduced. The effect of nitrogenous fertilisers on crop yield has been studied in many investigations, but there seem to be no data on the relative effects of early and late dressings except the following, reported in the "Notes from Craibstone" appearing in the April issue of the Scot. J. Agric. 1932. The plots received 1 cwt. ammonium sulphate per acre (1) when the crop was sown, (2) when the crop brairded and (3) three weeks later. The yields of grain were respectively 21.2, 22.0 and 20.8 cwt.; the yields of straw 42.2, 44.5 and 35.1 cwt. These results "are in agreement with previous trials, and indicate that a late application of nitrogen is less valuable than when applied earlier". Although the decrease in yield of straw seems to be fairly definite and agrees with observations made in the above 1934 trials, the yields of grain are essentially the same. It may, therefore, be assumed, meantime, that the yield of grain is not seriously affected by time of dressing so that the value of delaying the application for the purpose of increasing the nitrogen content of the grain would seem to be established.

(d) The type and amount of nitrogenous fertiliser. Several studies on the nutrition of the oat plant have dealt with the source of nitrogen. In his investigation, previously mentioned, Gericke (12) found certain minor differences between the effects of sodium nitrate and ammonium sulphate: they were principally in the yield of dry matter and most likely due to the time required for the nitrification of the ammonium salt. Stahl and Shive (28) observed that, in culture solutions, the rate of absorption of the ammonium and nitrate ions reached their respective maxima at the early stages and blossoming stages of the growth of the oat plant. Although/

Although their results would not necessarily apply to soils, they suggest that the relative effects of sodium nitrate and ammonium sulphate in the field would not differ materially, for under warm, moist conditions the ammonium ion is rapidly converted into nitrate. A spell of dry weather might, however, delay the effect of ammonium sulphate. Krüger, Wimmer and Lüdecke (18) compared various nitrogenous fertilisers and found that increased grain yields and nitrogen uptake by the grain varied in the same direction in the order $NaNO_3 > NaNO_3+(NH_4)_2SO_4 > NH_4NO_3$; but, on the other hand, Nehring (21b) found that the nitrogen intake from different sources was approximately the same.

In the series of experiments under consideration, only sodium nitrate and ammonium sulphate were used and only in 1931 were their relative effects compared. On that occasion there was no significant difference between the effects of the two salts when applied at the same time and supplying equal amounts of nitrogen. The smaller dressings, equivalent to 30 lb N per acre, did not affect the nitrogen content of the grain, the significant response to the nitrogen resting solely in the late double dressing. In 1930, 60 lb N per acre as sodium nitrate also gave very marked responses but in 1932, 1933 and 1934 definite responses were obtained with from 17 to 40 lb N per acre as ammonium sulphate. These are the two fertilisers most commonly used in this country for top dressings and, although sodium nitrate may give a quicker response, ammonium sulphate is a much cheaper source of nitrogen and its delayed action may be regarded as an added advantage when the object is to postpone the effect of the nitrogen.

With regard to the quantity of nitrogen which is required to produce the desired effect, it is necessary to keep in/

in mind what could be employed in practice without interfering seriously with the maturition of the crop. From the data available, it would appear that in a "late year", like 1929 or 1931, when the crop matures slowly and may be subject to serious lodging on account of heavy rains, only a large dressing would exert an effect. In other words, a normal dressing of 1 to 2 cwt. of ammonium sulphate, such as a farmer would not hesitate to apply, would be of no avail because the climatic factors would predominate. It is possible, of course, that the rather fertile soil on which the 1931 experiment was conducted also exerted an important influence, so that it is not safe to draw definite conclusions. When "early years", such as 1932, 1933 and 1934, are considered, on the other hand, the effects of from 17 to 40 lb. nitrogen per acre are quite striking. In many cases a dressing of that amount is commonly given at the time of sowing and could easily be delayed for 8 or 10 weeks without incurring additional expense in fertiliser or labour. Furthermore, the delay would give the grower an opportunity of deciding if the application of nitrogen was necessary or desirable to improve the tillering.

(e) The economic aspect. It appears, from seedsmen's valuations, that any differences in the nitrogen content of the grain are not apparent in its appearance and that they can only be assessed by chemical analysis. For the sake of simplifying the comparison of the results, as far as they concern nitrogen content of the grain, they are summarised below in tabular form, the nitrogen being expressed as a percentage of the mean in each experiment.

The/

The effect of nitrogenous fertiliser on the nitrogen content of the grain.

Year	Dressing in lb. N per acre	None	Early	Second	Late	S.E.	Diff. due to delaying of dressing.
1929	64	-	98.0	100.6	100.6	-	+ 2.6
1930	60	93.9	98.4	104.4	105.1	1.50	+ 6.7
1931	30	97.2	-	96.9	99.5	1.82	+ 2.6
"	60	97.2	-	-	107.7	1.82	-
1932	23	97.6	95.3	-	110.5	-	+15.2
1933	40	95.5	100.5	-	103.5	1.08	+ 3.0
"	40	98.4	97.9	-	104.3	0.99	+ 6.4
1934 (a)	23	-	98.6	-	101.4	-	+ 2.8
" (b)	17	-	92.5	-	107.5	-	+16.2
" (c)	23	98.0	99.0	-	102.7	-	+ 3.8
" (d)	23	100.9	96.9	-	102.1	-	+ 5.3

Although there must be some doubt about the significance of the differences in 1929, 1931, 1932 and 1934, the fact that the change is always in the same direction lends weight to the general conclusion regarding the effect of a late dressing. For the sake of obtaining an estimate of the possible value of the response, an average of the ten values, excluding the two very high ones, has been taken. It indicates that the average percentage increase in the nitrogen content of the grain, as a result of merely delaying the application of fertiliser from time of sowing until the beginning of June, is about 4.2. Since the average percentage of nitrogen in the grain was 1.91 in those seasons under consideration, the average increase in the actual percentage of protein was $\frac{4.2}{100} \times 1.91 \times \frac{100}{16} = 0.50$ (assuming that the crude protein contains 16 per cent. of nitrogen).

It is now possible to express that gain of protein in terms of shillings per ton of foodstuff on the basis of the farm value of oats as given monthly in the J. Min. Agric. In August 1934 (16), the cost per unit protein equivalent, calculated from imported barley, maize, decorticated ground nut cake and decorticated cotton cake, is 0.87 shillings. An increase of 0.50 per cent/

cent. protein is, therefore, equivalent to .50 × .87 shillings per 100 lb. oats or nearly 10/- per ton. Since the food value per ton of oats is about £5, the increase is equivalent to 10 per cent., a figure which must be regarded as of considerable economic importance.

This is really a very conservative estimate for, although the common conversion factor of 6.25 for crude protein is probably too high for cereals, the figure of 0.87 shillings per unit protein equivalent is the lowest for a long time: the farm value of oats has been fairly constant for the last 18 months but the price of starch equivalent has slightly increased recently whilst the price of protein equivalent has fallen very rapidly, so that it is now less than half of what it was in August 1933. An estimate of the monetary value of any food constituent is always determined by current market prices of foodstuffs, however, so that the figures given above cannot be otherwise expressed. Even at the present low value of plant protein, they seem to merit the attention of the farmer who grows an oat crop for home consumption. They also add weight to a recent suggestion (Hill, D.D., J. Amer. Soc. Agron. 1933, 25, 301) following upon a study of nearly 50 varieties grown in two years, that "protein testing of feed grains might be worth while" for the feeding value, as expressed by total and individual digestible nutrients, is frequently "not reflected in the statement of grade".

SUMMARY.

In a series of experiments and field trials extending over six growing seasons, a study has been made of the possibility of increasing the protein content of oat grain without interfering seriously with normal farm practice. A considerable variety of climatic conditions has been experienced in these seasons, so that the results taken as a whole are probably relatively independent of the variation in weather commonly met with in the East of Scotland area.

The method adopted has been to delay the application of nitrogenous fertiliser as late as it was practicable to broadcast it on the growing crop without permanent damage. The dressings have varied from 17 to 60 lb. nitrogen per acre and have been applied as late as the third week of June when the corn was over 18" in height.

Such characteristics of the grain as percentage of dry matter and "bushel weight" are not affected by the time of application of the nitrogen, nor is the valuation of the grain in the market. Attention is also drawn to the fact that the effect on the food constituents of the grain, other than protein, may be neglected. Ripening is retarded only to an unimportant extent and the susceptibility of the crop to lodge is not increased by delaying the dressing. From the available data, it may be inferred that, although the length of shaw is greatest when the nitrogen is applied about time of brairding, the yield of grain is not seriously affected by the time of application. In fact, the only disadvantage of the method is a possible small decrease in the amount of straw produced. In every experiment or trial, however, the percentage of nitrogen in the grain has been increased by delaying the nitrogenous dressing and a discussion of the results leaves little room for doubt that the effect is real and not/

not accidental.

From an average figure of 4.2 for the percentage increase in the nitrogen content of the grain and from the present extremely low price of unit protein equivalent, it is shown that the monetary value of the change amounts to an increase of approximately ten shillings per ton of oats.

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APPENDIX VII.

Latin Square. Analyses of grain of oats, 1930.

	Variance due to :-	Degrees of Freedom	Sum of Squares	Mean Square	z
Grain	Rows	3	.016	.0053	
Weight	Columns	3	.031	.0103	
	Treatments	3	.146	.0483	0.966
	Error	6	.042	.0070	
	Total	15	4.615	-	

Standard error for total of 4 plots $= \sqrt{4 \times .007} = 0.167$

Percentage	Rows	3	-	-	
Moisture	Columns	3	.233	.0776	
	Treatments	3	3.586	1.1953	1.0642
	Error	6	.854	.1423	
	Total	15	4.615	-	

Standard error for total of 4 plots $= \sqrt{4 \times .1423} = 0.754$

Percentage	Rows	3	.101	.0337	
Nitrogen	Columns	3	.074	.0246	
	Treatments	3	.156	.0520	1.378
	Error	6	.020	.0033	
	Total	15	.351	-	

Standard error for total of 4 plots $= \sqrt{4 \times .0033} = 0.115$

z is simply $1.1513 \times \log. \frac{\text{mean square for treatment}}{\text{mean square for error}}$

For $n_1 = 3$, $n_2 = 6$, the 1 per cent. point in the z table = 1.14 (1)

and the 5 per cent. point in the z table = 0.78

APPENDIX VIII.

Latin Square. Nitrogen in grain, 1931.

Plan.

The letter denotes the treatment, the figure is the percentage of nitrogen in the oven dried grain.

A = early dressing of ammonium sulphate at the rate of 30 lb.N per acre

B = " " " sodium nitrate " " " " " " "

C = late " " ammonium sulphate " " " " " " "

D = " " " sodium nitrate " " " " " " "

E = " " " " " " " " " 60 lb.N per acre

(1)

	Columns					Total
	D 2.53	A 2.50	C 2.45	B 2.43	E 2.90	12.81
	B 2.28	E 2.57	D 2.21	C 2.37	A 2.33	11.76
	C 2.29	B 2.24	A 2.23	E 2.24	D 2.27	11.27
	E 2.54	D 2.25	B 2.25	A 2.18	C 2.21	11.43
	A 2.11	C 2.34	E 2.37	D 2.29	B 2.18	11.29
Total	11.75	11.90	11.51	11.51	11.89	58.56
Difference from working mean	.10	.25	-.14	-.14	.24	

(2)

Treatments about a working mean of 2.33.

	A	B	C	D	E	Total	Mean
	.17	.10	.12	.20	.57	1.16	.232
	.00	-.05	.04	.12	.24	.11	.022
	-.10	-.09	-.04	-.06	-.09	- .38	-.076
	-.15	-.08	-.12	-.08	+.21	- .22	-.044
	-.22	-.15	.01	-.04	.04	- .36	-.072
Total	-.30	-.27	+.01	-.10	+.97	+ .31	.062
Mean	-.060	-.054	+.002	-.020	+.194	-	+.0124

To adjust treatment totals, add $5 \times 2.33 = 11.65$

Real totals	11.35	11.38	11.66	11.55	12.62	11.71

(3)

Squares of deviations from working mean.

	.0289	.0100	.0144	.0400	.3249	.4182
	0	25	16	144	576	761
	100	81	16	36	81	314
	225	64	144	64	441	938
	484	225	1	16	16	742
Total	.1098	.0495	.0321	.0660	.4363	.6937

Subtract product of grand total and general mean in (1) .003844

i.e. sum of squares of deviations for 25 plots = .689856

(4)

Sums of squares ascribable to :-

	Rows	Columns	Treatments
	1.3456	.0100	.0900
	121	625	729
	1444	196	1
	484	196	100
	1296	576	9409
Total	1.6801	0.1693	1.1139
Divide by 5	.33602	.03386	.22278
Subtract	.003844	.003844	.003844
Remainder	.332176	.030016	.218936

(5) Analysis of Variance

Due to :-	Degrees of Freedom	Sum of Squares	Mean Square	$\frac{1}{2}\log_e$ (Mean Square)
Rows	4	.332176	.083044	
Columns	4	.030016	.007504	
Treatments	4	.218936	.054734	2.003
Error	12	.108728	.009061	1.102
Total	24	.689856	-	

Standard error for total 5 plots $= \sqrt{5 \times .00906} = .213$ or 1.82 per cent. of the mean yield for 5 plots = 11.71.

For $n_1 = 4$ and $n_2 = 12$, the 1 per cent. point in the z table (1) is .844, so that the difference of .901 in the above table due to treatment is undoubtedly significant.

(6) Final result.

Treatment	A	B	C	D	E	Mean	Standard error
Per cent. N in grain	2.27	2.28	2.33	2.31	2.52	2.34	.213
As per cent. of mean	96.9	97.2	99.5	98.5	107.7	100.	1.82

Response to double dressing of sodium nitrate quite significant.

APPENDIX IX.

Randomised Blocks. Yield of oats, 1933.

Set A.

Block	Treatment 1	2	3	Total
1	14	12	15	41.5
2	11	15	14	40
3	10	14	15	39
4	12	14	16	42
5	15	14.5	13	42.5
Total	62	69.5	73	204.5
Mean	12.4	13.9	14.6	13.63

Standard error of mean of 5 plots = 0.72 but the effect of treatment, by the z test, is not significant.

Set B.

Block	Treatment 1	2	3	Total	Mean
1	10	9.5	12	31.5	10.5
2	7	11	10.5	28.5	9.5
3	10	10	11.5	31.5	10.5
4	9	11	13	33.0	11.0
5	11	12	(8)	23.0	
Total	47	53.5	(47)	147.5	
Mean	9.4	10.7			

According to Yates (2), the calculated value for block 5 treatment 3 is

$$x = \tfrac{1}{8}(3 \times 47 + 5 \times 23 - 147.5)$$

$$= 13.56$$

Substituting that figure for 8, the treatment means become 9.4, 10.7 and 12.1 and the standard error of the mean of 5 plots is 0.45

By the z test the treatment has exerted a significant effect.

Randomised Blocks. Nitrogen in grain, Set A, 1933.

Block	Treatment 1	2	3	Total	Mean	Standard error
1	1.89	2.06	2.02	5.98	1.99	
2	1.91	2.03	2.08	6.02	2.01	
3	1.81	1 98	2.01	5.80	1.93	
4	1.93	1.92	2.05	5.90	1.97	
5	1.93	1.95	2.09	5.97	1.99	
Total	9.47	9.94	10.25	29.66	9.8876	.106
Mean	1.89	1.99	2.05	General Mean,	1.9775	
Per cent.	95.5	100.5	103.5		100.0	1.07

Deviations from working mean = 1.98

1	-.09	.08	.04	.03
2	-.07	.05	.10	.08
3	-.17	0	.03	-.14
4	-.05	-.06	.07	-.04
5	-.05	-.03	.11	+.03
Total	-.43	+.04	+.35	- 04
Mean	-.086	+.008	+.070	General mean, -.0027

Grand total × general mean = .000,107

Total sum of squares of deviations = .0898

i.e. Sum of square of deviations from General Mean = .089,693

Blocks		Treatments	
Sum of squares of 5 totals	= .0303	Sum of squares of 3 totals	= .3090
Each 3 plots, i.e. divide by 3	= .0101	Each 5 plots, i.e. divide by 5	= .0618
Subtract	.000107	Subtract	.000197
Remainder	= .009993	Remainder	= .061693

Sum of squares ascribable to error = .089693 -(.009993 + .061693)

= .018007

Analysis of Variance.

Due to :-	Degrees of Freedom	Sums of Squares	Mean Square	$\frac{1}{2}\log_e$ (Mean Square)
1. Blocks	4	.009993	.002498	.4555
2. Treatments	2	.061693	.030847	1.714
3. Error	8	.018007	.002251	.4060
Total	14	.089693	-	-

For $n_1 = 2$ and $n_2 = 8$, the 1 per cent. point in the z table = 1.079

Hence the value 1.308 for treatments is clearly significant.

Standard error of single plot $= \sqrt{.002251} = .0475$ or 2.4 per cent.

" " " total of 5 plots $= \sqrt{.011255} = .106$ or 1.07 per cent.

Although the block "mean square" has accounted for a substantial amount of the soil heterogeneity, it is little more than the error "mean square", so that the influence of blocks is not greater than sampling error.

Nitrogen in grain, set B, 1933.

Analysis of Variance due to	Degrees of Freedom	Sums of Squares	Mean Square	$\frac{1}{2}\log_e$ (Mean Square)
1. Blocks	4	.011827	.002957	.542
2. Treatments	2	.045880	.022940	1.568
3. Error	8	.014053	.001757	.282
Total	14	.071760	-	-

z for 2,3 = 1.286, hence effect of treatment undoubtedly significant.

Standard error of total of 5 plots $= \sqrt{.001757 \times 5} = .0937$

Mean total of 5 plots $= 9.4800$

Hence percentage standard error $= 0.989$

The mean square for blocks is not significantly greater than the error mean square, even the 5 per cent. point in the z test for $n_1 = 4$, $n_2 = 8$ being equal to .6705.

Randomised Blocks. Grain weight and percentage moisture in grain, Set A, 1933.

	Weight of 100 grains in g. × 100				Percentage moisture in grain × 100			
	Treatment				Treatment			
Block	1	2	3	Mean	1	2	3	Mean
1	333	305	291	310	1143	1175	1182	1167
2	342	309	307	319	1130	1141	1147	1139
3	312	293	295	300	1162	1158	1167	1162
4	285	304	333	307	1180	1166	1175	1174
5	342	306	304	317	1159	1163	1181	1168
Total	1614	1517	1530	1554	5574	5803	5852	17429
Mean	323	303	306	311	1155	1161	1170	1162

Grain weight.

Analysis of Variance due to	Degrees of Freedom	Sums of Squares	Mean Square	$\frac{1}{2}\log_e$ (Mean Square)
Blocks	4	.073626	.018407	
Treatment	2	.110893	.055447	.856
Error	8	.287974	.035997	.641
Total	14	.472493	-	

Standard error of total of 5 plots = $\sqrt{.179985}$ = .422 or 13.1 per cent.

No significant difference due to treatment or blocks.

In the case of moisture in the grain, the mean values for treatment are all so close to the general mean that, even if the standard error were as low as 1 per cent., the differences due to treatment would be quite insignificant.

Randomised Blocks. Grain weight and percentage moisture in grain, Set B, 1933.

	Weight of 100 grains in g. × 100				Percentage moisture in grain × 100			
	Treatment				Treatment			
Block	1	2	3	Mean	1	2	3	Mean
1	315	325	308	316	1188	1210	1179	1192
2	310	309	330	316	1176	1179	1167	1174
3	321	317	361	333	1165	1171	1169	1168
4	308	320	325	318	1188	1180	1162	1177
5	318	298	326	314	1180	1156	1165	1167
Total	1572	1569	1650	1597	5897	5896	5842	5878
Mean	314	314	330	319	1179	1179	1168	1176

Grain weight

Analysis of Variance due to	Degrees of Freedom	Sums of Squares	Mean Square	$\frac{1}{2}\log_e$ (Mean Square)
Blocks	4	.071427	.017857	
Treatment	2	.084360	.042180	.720
Error	8	.133573	.016697	.256
Total	14	.289360	-	

Hence there are no significant differemces due to treatment.

Standard error of total of 5 plots = $\sqrt{.0835}$ = .289 or 9.2 per cent.

The moisture content of the grain is obviously not affected by treatment.

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An Examination of the Aspergillus niger Method of Soil Analysis.

INTRODUCTION.

The Manurial Requirement of the Soil. Until comparatively recently, the use of fertilisers has been based very largely upon trial and error demonstrations, the appearance of the crop under different treatments usually receiving greater attention than yield or quality. When one considers that even the most expert observer can seldom with confidence distinguish growing crops differing by less than 10 per cent. in yield, it is obvious that important differences may easily fail to be detected by visual examination alone. Innumerable yield trials are, of course, carried out but, unfortunately, very often without due regard to the errors involved. Well established and properly conducted field experiments are comparatively few in number and the conclusions reached from them necessarily apply rigidly only to the particular conditions of soil, environment and so on in question. It is obviously impracticable to carry out an unlimited number of such experiments and much attention has naturally been devoted to studying the manurial requirements of the soil under the less satisfactory laboratory conditions. The problem which has confronted the investigator is the question of the availability of nutrients required by the plant. It is well known that plants may respond to additions to a soil of certain elements necessary for growth, although the soil contains large amounts of those elements. Discrimination between available and non-available constituents is very difficult, partly on account of the heterogeneity of the soil and its ever-changing character and partly because the feeding power of plants is still a matter of controversy.

The Availability of Plant Nutrients. Many purely chemical methods have been proposed and adopted. Generally speaking, they involve an extraction of the soil with water or some/

some dilute acid and aim at estimating the quantities of those substances which would normally come into solution under field conditions as a result of chemical and biological changes and the effects of plant growth. In certain circumstances, when the results can be properly interpreted in the light of experience, the information obtained is of the greatest value for advisory purposes; but all chemical methods must suffer from serious defects. The results depend upon the technique, so that strict comparison under different conditions is difficult and frequently impossible; the results necessarily give only a measure of the state of the soil at a particular time and obviously cannot apply to its conditions before, during and after cropping; no chemical method can imitate the lengthy and complicated process of assimilation of nutrients by the plant which undoubtedly plays an active part itself in helping to bring them into assimilable form. There is probably no hard and fast line between that part of a constituent which is available to the plant and that part which is not; the compounds of the soil are continually undergoing change and even the minerals and organic residues most resistant to decomposition are gradually broken down to supply plant food.

These fundamental difficulties have persuaded investigators to turn to the plant itself for information regarding the availability of nutrients and the manurial requirements of soils. This has given rise to an intensive study of the growth and composition of plants in various types of dish and pot experiments, with a view to determining what is easily available to plant and what constituents are lacking in the soil. Mention only need be made of the Mitscherlich and Neubauer methods of estimating manurial requirements, methods which are used widely and successfully on the Continent. Both methods are open to criticism because the soil is examined under artificial conditions and

there/

there are also certain objections on theoretical grounds; but there is no doubt that they are the best available methods for obtaining an estimate of the amount and composition of fertiliser required to improve the cropping power of the soil. One disadvantage of both methods is that they are not suitable for general use except by Institutes specially equipped and staffed for the purpose. The Mitscherlich method is costly and requires a whole growing season to obtain results, while the Neubauer method demands constant supervision and skilled analyses. Consequently, for the ordinary laboratory, which may be required to report on some hundreds of samples of soil annually, efforts have been directed towards finding a cheaper, simpler and more rapid method for routine purposes.

Biochemical Methods. It has been observed that the development of certain lower organisms may form a good index of soil fertility; in other words, conditions which are satisfactory for the growth of higher plants are also usually suitable for some lower organisms. It is, therefore, possible to assess the cropping power of the soil by studying the development of a bacterium or fungus in the soil under suitable conditions, and, since the growth of these organisms is rapid, results are obtained in a few days. The possibilities of such a method have been known for many years, but its intensive study and practical application have been taken up only recently. For the estimation of manurial requirement, the method is based essentially on the course of development of the micro-organism in a culture medium containing a definite amount of soil in place of the nutrient in question. Generally speaking, the higher plants and lower organisms react similarly to the different nutrients. Two types of organism have been employed for this purpose, viz. the bacterium Azotobacter chroococcum and the mould fungus Aspergillus niger.

Azotobacter/

Azotobacter is an important soil micro-organism which is able to fix atmospheric nitrogen, and its use in soil analysis originated in Denmark. It was observed, in the course of a series of liming experiments, that this organism developed when there was an adequate supply of bases in the soil but failed when the soil was acid. The sensitivity of the organism to acid conditions became the basis of a method of estimating the "lime-requirement" of the soil, but more accurate physico-chemical methods are now generally employed.

An adequate supply of phosphate is also necessary for the growth of Azotobacter and the content of easily available phosphate in the soil may be estimated by studying the development of the organism under appropriate conditions. Unfortunately, the rate of development of the organism can only be estimated by the number and size of the colonies of cells which become visible to the naked eye after a short period of incubation, so that there is considerable experimental error; but in Denmark, where the method is regularly used, it is claimed to be easy to grade the degrees of development into "poor", "moderate" and "good", corresponding respectively to large, moderate and small soil requirements of phosphate.

The use of Aspergillus niger has developed more recently but is similar in principle to the Azotobacter method. The necessary conditions of growth of the two organisms are quite different, however, for whilst Azotobacter requires an approximately neutral medium, Aspergillus niger develops well in acid media, and whereas the degree of development of Azotobacter cannot be measured accurately, it is possible to collect the fungus and weigh it. It has no claim to being an accurate quantitative method but rather a means of linking up the more qualitative Azotobacter method with the Neubauer and various chemical methods, its great advantage/

advantage over the latter being simplicity and speed for routine analyses.

The name "Aspergillus niger" is applied to a group of those fungi commonly known as moulds. The genus Aspergillus is characterised by the interlacing of tubular filaments to form a vegetative felt, called a mycelium, which is coherent and easily handled. Reproduction takes place in various ways but, in that which chiefly concerns us, large numbers of conidia or spores are produced. The conidia, each of which is capable of developing into a new plant, are almost black in colour and are responsible for the dark velvety appearance which is typical of the fungus.

In comparative work it is essential that a standardised technique be followed because the weight of mycelium depends upon the amount and relative proportions of food material available. If the concentration of the solution be too great, the growth proceeds for an inconveniently long time, whereas if the concentration be too low, the weight of mycelium formed is too small since it is closely related to that nutrient which is the limiting factor. The culture solution, containing all the constituents necessary for growth except that for which the soil is being tested, also contains 1 per cent. citric acid. This ensures a strongly acid reaction which is favourable for the growth of the Aspergillus and reduces the disturbing influence of other organisms.

The mixture of soil and culture solution is inoculated with the organism and incubated for from 4 to 6 days according to the object of the experiment. During that time the mycelium develops and covers the surface of the liquid like a felt. When the growth is large, the surface presents a corrugated appearance and is tough and almost rubber-like to touch. The mycelium is removed/

removed from the flask, washed with water to free it from any adhering soil particles, dried slowly in an oven and weighed. During growth, the acidity of the suspension increases to a certain extent owing mainly to utilisation by the fungus of the nitrogen of the ammonium salt present with liberation of free mineral acid. The change depends upon the rate of development of the organism and the chemical nature of the soil. It is not possible to control the acidity during growth so that, since the amount of nutrient which becomes available is related to the degree of acidity of the solution, it is obvious that, the more active the growth of the organism, the better will become the conditions for setting free the limiting nutrient. Consequantly, the differences between soils will tend to be exaggerated since the final degree of acidity may lie between fairly wide limits. (In this connection, it may be mentioned that proposals have been advanced by Butkevitch (1) for the use of Aspergillus oryzae which is claimed to develop less acidity.) If, under certain conditions, the growth is allowed to proceed too long, then the protein compounds of the mycelium break down with liberation of ammonia; this reduces the acidity of the medium to a point favourable for other micro-organisms and the Aspergillus may be decomposed to such an extent that it cannot be collected. The addition of citric acid to the culture solution and the time growth are, therefore, two factors of the utmost importance in the method.

The results are also affected by the particular strain of the organism used and by the temperature of incubation which must be carefully controlled. Unless all these factors are maintained nearly constant, it is quite possible for different soils containing the same amounts of potassium or phosphorus to give quite different results. The variations which occasionally occur/

occur in spite of the above precautions are undoubtedly a weakness in the method because uniformity of results is essential for any satisfactory method of investigation. No method, however, depending upon living organisms which do not obey strict chemical laws can be expected to provide perfectly constant results; the success of the Aspergillus method must therefore be circumscribed by certain reservations. Studies in the behaviour of the organism are being made at various places and the interpretation of the results will become more rigid when all the variable factors have been thoroughly investigated and adequate comparison made with other data.

There is a certain similarity between the Aspergillus method and chemical methods which make use of citric acid to bring easily available nutrients into solution; one difference, however, is that in the biochemical method one nutrient is being examined in presence of excess of all the others, whereas in the purely chemical methods the proportions of the nutrients present in the soil remain unaltered. As a matter of fact, the growth of Aspergillus is not so great when a citric acid extract of the soil is used in place of the soil itself and the results do not agree so well with those from the Neubauer method. That is to say, more nutrient is withdrawn from the soil during the growth of the organism than by the extraction with citric acid. This might be expected since the acid extraction gives us the amount of nutrient which is dissolved at room temperature under definite experimental conditions. The value obtained in the Aspergillus method, on the other hand, corresponds to the supply of nutrient which becomes available during the vegetation period when the temperature is higher, the acidity becomes greater and the plant nutrients are undergoing constant change.

Applications of the Aspergillus Method. Many papers dealing/

dealing with the application of this biochemical method of estimating the available plant food in the soil have been published in the course of the last few years. The development of different strains of the fungus and its behaviour in various culture solutions have been studied, and the work has been extended to the investigation of soils. Hundreds of results have been submitted to show the agreement between this method and other standard methods (especially the Neubauer method) for estimating the fertility and potash or phosphate requirement of soils. The comparative success of the investigations has justified the emphasis which has been laid on the merits and general usefulness of such a simple and cheap method for rapid routine analysis (14). No claim has been made for infallibility or accuracy, but the usefulness of a simple test, which can place the majority of soils into three or four groups, according to their degree of fertility, cannot be overrated. It has been severely criticised by Stock (17) on the theoretical grounds that so many factors are not under control and the point of maximum growth is uncertain, but even those workers who have been most prominent in advocating its merits have accepted its limitations and emphasised its usefulness simply as a convenient routine test, which probably falls between the purely chemical and the biological methods. The value of the accurate chemical determination is always open to question when proper consideration is given to the difficulties and errors associated with the sampling of the soil, the distribution of fertilisers and the interpretation of the results. In the biological method there must always be factors, internal or external, which fall outside experimental control. The field experiment is naturally the only satisfactory test of the precision and reliability of any method. As already mentioned, it can be practiced only to a limited extent, but is absolutely necessary/

necessary to support the claims for any laboratory method, and the mere comparison of different laboratory methods is of second rate importance.

Meantime, therefore, until there is more general agreement regarding the best standard to adopt, it is highly desirable to examine any method which may prove to be useful for advisory purposes. Even if it only serves to tell that the suuply of potassium or phosphorus in a soil is good, moderate or poor, it is valuable for the rapid examination of the large numbers of samples with which many laboratories have to deal. The Aspergillus method would seem to offer such a chance, and the work carried out in this investigation was designed primarily to examine some of the more important factors concerned in the laboratory technique.

1. METHOD.

The technique which has been employed throughout differs only in minor details from that adopted by the investigators at Weihenstephan (8, 20). The preparation of the suspension of conidia has been simplified; ammonium nitrate has been used in place of ammonium sulphate; squat, wide necked bottles have been found more convenient than conical flasks. Ordinary conical flasks, of 125 c.c. capacity and 6 cm. diameter, were first used, but some difficulty was experienced in removing large growths of mycelium through the rather narrow neck, and they were later replaced by bottles of 130 c.c. capacity, 5.5 cm. diameter of base and 4 cm. diameter of neck. These have been found to be extremely satisfactory for they take up much less room than flasks in an incubator, withstand comparatively rough treatment and are easily washed. See photographs 4 and 5.

For the estimation of "available potash", 2.5 g. soil

are/

are placed in a bottle and 30 c.c. of a nutrient solution, having the following composition, are added from a pipette:-

10% sucrose, 1% citric acid. 0.1% peptone, 0.075% P_2O_5 (as ammonium dihydrogen phosphate), 0.36% ammonium nitrate, 0.03% magnesium sulphate, 0.00015% copper, 0.0001% iron and 0.0001% zinc (each in the form of sulphate).

A few drops of a suspension of conidia (see below) are then added and the whole incubated for six days at 35°C. The mycelium is then removed from the bottle by means of forceps, washed in water, placed on a tared clockglass and dried, first overnight at 50-60°C., then for two hours at 60-100°C. and finally for two hours at 100-105°C. It is then cooled in a desiccator and weighed. For routine purposes, the experiment is carried out in quadruplicate, the four mycelia being dried and weighed together on the same clockglass and the average weight obtained by calculation.

In the estimation of "available phosphate", 5 g. of soil are taken instead of 2.5 g., while the nutrient solution contains 0.02% K_2O as potassium sulphate and no phosphate. Otherwise the technique is the same as above. In the following sections dealing with an examination of the factors (a) inoculation, (b) source of nitrogen, and (c) size of vessel, nutrient solutions containing excess potassium but no phosphorus have been employed.

2. INOCULATION.

Fresh cultures of the organism are prepared from time to time on slopes of agar containing 1% glucose, 0.1% asparagine and 0.05% K_2HPO_4 in tap water. A small quantity of the conidia is carefully removed from the surface of the slope, by means of a moist wire loop, and shaken up with a few c.c. of distilled water. Three drops of the suspension are added to each bottle. This

method/

method is simple compared to the carefully controlled measures recommended by Niklas and his co-workers, but experiments have demonstrated that it is sufficiently accurate for the purpose.

The following figures (Table 1a), obtained in a preliminary experiment with various dilutions of a suspension of conidia, illustrates that a 90-fold difference has little or no effect.

Table 1a.

Average weights of mycelium in g. obtained with different concentrations of inoculum.

Relative concentration of inoculum	1	3	10	30	90
Soil Wo	0.31	0.31	0.30	0.30	0.32
Au	0.44	0.46	0.45	0.44	0.44
Cr	0.86	0.86	0.87	0.87	0.84

Although the slope cultures might be uniform in age and appearance and the technique as constant as possible, it was felt that the above range of concentration was rather small to permit of definite conclusions. The following figures (Table 1b), however, show that the differences in mycelium weight, obtained over even a million-fold range in concentration of inoculum, are negligible.

Table 1b.

Average weights of mycelium in g. obtained with different concentrations of inoculum.

Relative concentration of inoculum	1	10	10^2	10^3	10^4	10^5	10^6
Soil 1	.34	.35	.35	.34	.36	.37	.36
2	1.23	1.15	1.15	1.14	1.17	1.28	1.24
3	.27	-	-	.31	-	-	.31
4	.45	-	-	.43	-	-	.42

3. SOURCE OF NITROGEN.

One of the inherent objections to the Aspergillus niger method is the change in acidity which takes place during growth as/

as a result of the absorption of cations from the nutrient solution and the liberation of the corresponding mineral acids. This is particularly important in the estimation of available phosphate in the soil and is no doubt partly responsible for the fact that the method is better adapted to the estimation of the potash requirement than of the phosphate requirement of soils. Several investigators (3, 4, 20) have examined the question of change in acidity but chiefly with nutrient solutions varying in composition but containing ammonium sulphate as the source of nitrogen. Since the bulk of the free sulphuric acid liberated must come from the ammonium sulphate it was thought that either ammonium citrate or ammonium nitrate might prove to be more suitable in this respect. The liberation of citric acid from ammonium citrate would not sensibly affect the acidity of the solution which already contained 1% citric acid. It has, in any case, been shown by Trischler (20) and by Niklas et al (9) that a considerable variation in the quantity of citric acid present does not affect the weight of mycelium formed nor the final pH value to any extent. It has also been shown by Lowig (4) that, as far as the source of potassium for the fungus is concerned, the citrate anion behaves exactly like chloride, sulphate and silicate. The acid salt of ammonium citrate, $(NH_4)_2C_6H_6O_7$, has been used by Wehmer(24) in the study of the formation of organic acids by A. niger, but the normal salt, $(NH_4)_3C_6H_5O_7$, has been used in the experiments described below.

Although ammonium sulphate seems to be generally regarded as the best source of nitrogen for the organism, Simakova and Bovschik (12) have used ammonium nitrate with success in the study of soils. Certain results have also been obtained, by Wehmer (23), on solutions, showing that A. niger develops as well with ammonium nitrate as with sulphate at temperatures of

32-34°C/

32-34°C. and that the rapid increase in titratable acidity produced in the early stages of growth is rather less with the former salt. This may be regarded as due to the fact that the A. niger can utilise both nitrate and ammoniacal-nitrogen. Consequently, it might be assumed that the changes in acidity would be less with ammonium nitrate and the results more strictly comparable for different soils.

Table 2 contains the average results obtained with a number of soils.

Table 2.

Effect of source of nitrogen on mycelium weight and acidity.

Ammonium salt	Mycelium	pH of soil suspension at beginning	pH of soil suspension after 6 days
Sulphate (11 soils)	0.51	2.66	2.60
Citrate (")	0.61	4.10	3.08
Sulphate (6 soils)	0.60	2.85	3.02
Nitrate (")	0.62	2.82	3.15

There was no apparent relationship between the final acidity of the suspension and the mycelium weight for the different soils with any of the nutrient solutions.

The differences between sulphate and nitrate were very small but, in view of the tendency for the latter to give greater weights of mycelium, there seemed to be some justification for employing nitrate in place of sulphate in subsequent work.

The acidity of the citrate solution did not remain reasonably constant and the initial acidity was not great enough to prevent the development of other organisms in addition to the Aspergillus. Although the average weight obtained was higher with citrate than with sulphate, the growth was not so satisfactory, and on occasion there was considerable bacterial development with citrate. The latter disadvantage might be obviated by sterilisation but, in order to keep the method as simple as possible, it was thought that the initial pH of the nutrient solution/

solution containing citrate might be lowered sufficiently by increasing the concentration of citric acid. As already mentioned, it has been shown (9, 20) that a considerable variation in the quantity of citric acid present may not affect the weight of mycelium formed nor the final pH value to any appreciable extent. Three nutrient solutions (A, B and C), containing equal amounts (0.75%) of normal ammonium citrate but with 1, 1.3 and 2.2% citric acid respectively, were, therefore, compared with the usual ammonium nitrate nutrient solution. The results are summarised in table 2a.

Table 2a.

Weight of mycelium and final pH of suspensions with different solutions.

Solution	Nitrate	Citrate A	Citrate B	Citrate C
Original pH value	2.26	3.81	3.63	3.13
Soil 5	.32 (2.68)	.49 (3.21)	.52 (3.04)	.60 (3.07)
6	.42 (2.79)	.65 (3.11)	.68 (2.83)	.67 (2.72)
7	1.23 (3.25)	1.23 (3.09)	1.28 (2.95)	1.25 (3.02)

The figures in brackets show that the citrate solutions gave a rather more constant final pH value for the different soils than the nitrate solution. The differences in yield between soils are much reduced, however, and that is a serious disadvantage. It was decided, therefore, to adhere to the use of ammonium nitrate as a source of nitrogen for the organism.

4. SIZE OF VESSEL.

As might be expected, the weight of mycelium formed depends to a certain extent upon the area of surface upon which growth takes place. Different workers seem to have used, almost exclusively, small flasks of base diameter about 6 cm., and only brief mention has been made of the effect of vessels of other size and shape. The following figures (table 3) for a series of soils using beakers, flasks and bottles differing, in cross section,

will/

will give an idea of the nature of the results which may be obtained. In each case a diameter of approximately 6 cm. has been taken as standard. The depth of suspension varied because the amounts of soil and liquid were constant throughout.

Table 3.

Variation of mycelium weight with surface area of growth.

Diameter of vessel (cm)	Relative cross section	Relative weights of mycelium				Ratio mycelium ÷ cross section			
		Soil W	C	G	L	W	C	G	L
3	25	44	39	39	71	176	156	156	284
6	100	100	100	100	100	100	100	100	100
9	225	150	166	154	171	67	74	68	76

The actual weights of mycelium which were obtained with the above soils in bottles of 6 cm. diameter will be found in table 4. It will be observed that the relative weight of mycelium per unit cross section has been increased by about 60 per cent. by reducing the relative area of cross section from 100 to 25, and decreased by about 30 per cent. by increasing the relative area of cross section from 100 to 225. The results for the very fertile garden soil L are exceptional. The limits of size chosen considerably exceed those which would normally be employed in practice, for a diameter of 3 cm. is too small and one of 9 cm. is too large for ordinary purposes. A vessel of about 6 cm. diameter is very convenient for the quantities of soil and solution commonly employed; some additional experiments were carried out, however, with different sized vessels using different quantities of soil and culture solution, to compensate for different depths of suspension, but always in the ratio of 1 g. to 6 c.c.

Table 3a./

Table 3a.

Diameter of vessel (cm.)	Relative area of cross section	Soil (g.) + solution (c.c.)	Weights of mycelium Soil 3	5	6
3.75	46	2.5 + 15	.16	.17	.26
		5 + 30	.17	.13	.19
5.5	100	5 + 30	.28	.31	.40
		10 + 60	.27	.26	.40
8.0	212	5 + 30	.88	1.16	1.28
		10 + 60	.90	.92	1.15
		20 + 120	.90	1.11	1.12

The results (table 3a) indicate that the area of growth is a much more important factor than the actual amount of suspension and that, in comparative work, it is desirable to use vessels whose diameters do not vary by more than a few mm.

5. THE EFFECT OF STRAIN OF THE ORGANISM.

The question of the effect of different strains of the organism and of the age of the cultures when used, on the formation of acid in nutrient solutions has been the subject of several investigations (3). A summary of the results of a study of four strains of the organism on a number of soils, differing considerably in their properties, are submitted below (table 4).

Table 4.

Estimation of available phosphorus.
Influence of strain of A. niger on mycelium weight.

Strain	1. Edinburgh		2. Boas		3. Boas-Poschenrieder		4. Indian	
	myc.[1]	var.[2]	myc.	var.	myc.	var.	myc.	var.
Soil X	514	1415	548	393	436	570	463	772
Y	578	294	579	1034	552	149	615	771
L	1087	254	1184	2979	1162	973	1191	1268
mean	726	-	770	-	717	-	756	-
W	316	149	268	568	260	663	-	-
C	393	149	421	857	407	125	-	-
B	416	110	447	2016	445	593	-	-
G	579	836	672	1939	591	1390	-	-
A	533	2572	534	1065	495	785	-	-
S	1038	728	1037	200	1054	156	-	-
L	1201	3782	1157	360	1182	901	-	-
mean	639	-	648	-	633	-	-	-

[1] mycelium weight represents mean of 12 replicate observations in mgm.
[2] variance = sum of (deviations from mean)^2/(n-1)
where n = number of observations = 12.

The technique was as already described for the estimation of available phosphate, (sections 1 and 2), except that 12 observations were made for each soil and each strain in order to get a good estimate of experimental error. The original cultures of the different strains were kindly supplied by Professor Niklas and Dr. Poschenrieder of Weihenstephan and by Dr. Wilson of Edinburgh.

Every reasonable precaution was also taken to avoid differences in experimental error within and between treatments. Each batch of tests consisted of 36 samples of the same soil in three series of 12 for the 3 strains. No two batches were in the incubator during the same 6 days, but the same sections of the incubator were used throughout the investigation and the temperature remained constant at $34.5 \pm 0.5^{\circ}C$.

The results from the first three soils, X, Y and L have been analysed separately from the others because they were obtained about nine months earlier with a nutrient solution containing ammonium sulphate as a source of nitrogen. It is noteworthy that the results for soil L in the two sets show very good agreement. The general mean for the first three soils and four strains is 742.5 and the standard error of the mean of 12 observations is 1.2 per cent.

It will be observed that sometimes one strain and sometimes another gives the highest result for the different soils but that the mean values for the different strains are fairly uniform. It, in fact, appears as if there might be a significant interaction between strain and soil or, in other words, that the strains give different results with different soils. There is no apparent relationship between the mean value and variance for the different soils and strains, which indicates that the variability is not regular.

The results/

The results for the last seven soils have been analysed statistically and the observed variance of variance (based on 20 degrees of freedom) is 881,958. Assuming that the variation in variance is due to random errors, this theoretical value should be $2 \times (\text{mean variance})^2 \div 12 = 2 \times 950^2 \div 12 = 150,417$ based on $11 \times 21 = 231$ degrees of freedom. The value of $\underline{z} = \frac{1}{2} \log_e 5.86 = 0.88$, is quite significant so that the variability is not homogeneous.

The analysis of variance for the above set of results is as follows :-

Due to :-	Sum of Squares	Degrees of Freedom	Mean Square	$\frac{1}{2} \log_e$ (Mean Square)
1. Strains	9,576	2	4,788	4.236
2. Soils	24,970,752	6	4,161,792	-
3. Residual	105,768	12	8,814	4.540
4. Total (between treatments)	25,086,096	20	1,254,305	-
5. Within treatments	219,994	231	952	3.430
Total	25,306,090	251	-	-

(a) $\underline{z}$ for 1, 3 = 0.304; 5 per cent. point in $\underline{z}$ table for $n_1 = 12$, $n_2 = 2$ is 1.48

(b) $\underline{z}$ for 3, 5 = 1.110; 1 per cent point in $\underline{z}$ table for $n_1 = 12$, $n_2 = 231$ is 0.39

Standard deviation $= \sqrt{952} = 30.85$

i.e. Standard error (of mean of 12) $= 30.85/\sqrt{12} = 8.906$

Percentage standard error (General mean = 640) = 1.39

(Standard error of mean of quadruplicate observations = 2.41 per cent.)

In the first four rows of the above table, the actual sums of squares are concerned with the results between treatments and have tbeen multiplied by 12, the number of tests in each treatment, in order that they may be compared with the results within treatments. An extremely large amount of the variation

is/

is, as would be expected, due to soils, but that is of no direct importance in the examination of the results. The mean square for strains is actually less than that for the residual error between treatments so that, according to these results, strain does not exert a specific effect. That statement is in keeping with the earlier observation that the mean values for the different strains were fairly uniform.

The variation within treatments is very much less than the residual errors between treatments. There may be some uncertainty about the exactness of the test of significance on account of the heterogeneity of the variance, but the variance ratio is so far above the .01 level that the reality of this interaction between soil and strain is scarcely to be questioned. The possibility, suggested by a study of the results in table 4, of different strains putting a series of soils in different orders is, therefore, amply proved by the complete examination of the data.

The question is perhaps mainly of academic interest meantime in view of the lack of knowledge on the reliability of the Aspergillus method in practice. A programme of co-operative work, on the estimation of soil fertility, has recently been adopted by the International Society of Soil Science (19), however, and it is obviously important that any co-operation on the Aspergillus method would require a careful consideration of the variability of the strains of the organism. It was decided, therefore, to examine the same seven soils for available potassium, using three of the same strains. The results are summarised in table 4a.

Table 4a./

Table 4a.

Estimation of available potassium.

Influence of strain of A. niger on mycelium weight.

Strain	1. Edinburgh		2. Boas		3. Boas-Poschenrieder	
Soil	Weight[1]	Variance[2]	Weight	Variance	Weight	Variance
W	284	187	246	216	297	227
C	292	920	287	118	355	145
B	372	721	369	415	470	686
G	315	548	263	138	320	202
A	240	40	236	248	231	50
S	243	118	202	73	238	96
L	1038	191	958	881	1000	549
Mean	398		366		416	

General Mean = 393.1

[1] Mycelium weight represents mean of 10 replicate observations in mgm.

[2] Variance = sum of (deviations from mean)^2/(n-1) where n = 10.

Analysis of Variance.

	Sum of squares	Degrees of Freedom	Mean Square	$\frac{1}{2}\log_e$ (Mean square)
1. Strains	89,670	2	44,835	3.053
2. Soils	13,447,890	6	2,241,315	-
3. Residual	81,160	12	6,763	2.107
4. Total (between treatments)	13,618,720	20	680,936	-
5. Within treatments	60,921	189	322	0.584

(a) z for 1, 3 = 0.946 . (b) z for 3, 5 = 1.523

Standard error of mean of 10 replicates $= \sqrt{322/10} = 5.68$

Percentage error of mean of quadruplicate estimations $= \sqrt{\frac{322}{4}} \cdot \frac{100}{393}$

$= 2.28$

As before, there is no apparent relationship between the mycelium weight and variance for different soils and strains, and an analysis of the variance of variance (see p. 165) shows that the variability/

the variability is not homogeneous. It is necessary to keep this heterogeneity in mind in the examination of the results below.

The first four rows in the analysis of variance are concerned with the results between treatments, and the sums of squares have been multiplied by 10, the number of tests in each treatment, so that a final comparison may be made with the variance within treatments. The total number of tests is 21, each having 10 replicates. It will be observed that this time the mean square for strains is significantly greater than that for residual error between treatments, the ratio being exceeded by chance in only rather more than 1 per cent. of cases. The observed ratio of 0.946 is slightly less than that (0.968) in the 1 per cent. test for $\underline{z}$ ($n_1 = 2$, $n_2 = 12$) (2). In other words, strain exerts a specific effect. The Boas-Poschenrieder strain exhibits the least variability and has generally been employed in routine work. It is interesting to note that it was also the best strain in the estimation of available phosphate, although in that case the analysis of the data showed that strain did not have a specific action. That may suggest that the test for phosphate is less delicate than that for potassium but, on the other hand, the range of values obtained in the phosphate estimations was much greater than that for potassium.

The fact that the residual errors between treatments is again so much greater than the variation within treatments shows that there is a significant interaction between strain and soil. The 1 per cent. $\underline{z}$ test for $n_1 = 12$, $n_2 = 189$ gives a value of 0.4 against the observed ratio of 1.5 and, although it is not desirable to stress the exactness of the test on account of the heterogeneity mentioned above, the level is such as to leave little doubt of the reality of the effect. It is not improbable that these effects would be much greater with other strains of the organism and it is obvious/

obvious that considerable caution is called for in the more general comparison of results of different workers.

6. LABORATORY ERROR.

Little need be added on this question to the figures already given. With the strains examined, the standard error of the mean of twelve estimations of potassium or phosphorus in any soil is approximately 1.4 per cent.; the limits have been 0.3 and 3.3 per cent. and, as previously mentioned, there is no definite linearity between variances and mean values. For routine purposes, the tests are usually made in quadruplicate and the corresponding figure is 2.3 or 2.4. These figures are less than those reported elsewhere (9) and suggest that the accuracy of the method, in regard to laboratory technique, is all that could be desired when the limitations of the agreement so far recorded between laboratory and field results are taken into consideration.

7. EFFECT OF SOIL REACTION.

In view of the necessity of having sufficient acid in the nutrient solution to prevent the growth of various soil micro-organisms, the initial reaction of a soil may have little influence upon the initial or final pH value of the suspension. When more than 1 per cent. of calcium carbonate is present in the soil, it is neutralised by the addition of an extra amount of citric acid (8). Considerable stress, however, has been laid upon the effect of calcium salts upon the development of Aspergillus niger. Figures have been given (10), for example, to show the influence of additions of calcium carbonate to the nutrient solution in the estimation of available potash in soils; the average weights of Aspergillus obtained from calcium carbonate-free soils by the addition of 0.0, 0.1, 0.25, 0.5 and 0.75 per cent. of $CaCO_3$ to the nutrient solution were respectively 368, 420, 414, 509 and 589 mgm. These data, together with a large

number/

number of observations made with soils containing free calcium carbonate demonstrated that the presence of calcium carbonate increased the apparent available potassium of the soil. Less information is available with respect to the influence of calcium on the phosphate in the soil, but, according to Vilsmeier (22), the presence of calcium carbonate increases the absorption of phosphorus and calcium by the mycelium and also the mycelium weight, to a smaller extent. The need of the correction for calcareous soils, recommended by Niklas and Poschenrieder, however, has been disputed (5) and it is probable that the influence of lime depends largely upon the initial degree of saturation of the soil.

The following results have been obtained with two soils from a series of pot experiments. Soil W is an extremely infertile sandy loam which was treated with increasing amounts of calcium hydroxide in 1931. Soil B is a normal clay loam one sample of which was treated in 1930 with calcium hydroxide to decrease the acidity and another sample of which was treated annually with sulphur to increase the acidity. The average figures obtained from samples taken in February, 1933, from 70 pots, are given in table 5.

Table 5./

Table 5.

The effect of soil reaction upon the development of A. niger.

Soil	$Ca(OH)_2$ added to soil per cent.	pH	Weight of mycelium in estimation of K (1)	K (2)	P_2O_5 (1)	P_2O_5 (2)	Average relative crop yields.[3]
W1	0.00	4.5	271	254	273	303	10
W2	0.20	5.4	262	275	348	335	56
W3	0.45	6.3	249	279	351	326	96
W4	1.00	7.9	272	278	332	354	104
B1	(sulphur)	5.4	302	398	405	395	99
B2	0.00	6.0	299	389	412	422	100
B3	0.12	7.0	301	412	410	432	97

1 After 2 years cropping in set W, 3 crops in set B.

2 " " " fallow " " ", 3 years " " "

3 Oats, peas, beans and wheat in the case of Soil W;
potatoes, barley, peas, beans and wheat in the case of Soil B.

Soil W has an extremely low degree of saturation and contains a fair amount of exchangeable aluminium and iron (see p. 21). The addition of lime has had no effect on the available potassium in the cropped soil, as estimated from the development of A. niger. It has effected an increase, however, in the uncropped pots. The addition of lime has brought about significant increases of available phosphate in both cropped and uncropped soils and it is noteworthy that the cropping has reduced the available phosphate in the unlimed soil. It may be added that in an additional set of pots containing the same soil, the application of a concentrated soluble fertiliser (8% N, 16% P_2O_5, 16% K_2O at the rate of 400 lb. per acre) did not increase the crop yield, and the weight of mycelium obtained in the estimation of available potassium was 314 mgm. The application of sufficient lime to bring the pH to 7.7 in addition to/

to the fertiliser, gave a mycelium weight of 303 mgm. and increased the crop yield tenfold. These figures are significantly higher than that for the unlimed and unfertilised soil, although not different from each other. In the estimation of available phosphate, the corresponding mycelium weights were 268 and 376 mgm. which indicated that the soluble phosphate added to the soil was rendered unavailable unless calcium was also applied. This acid infertile soil is, of course, rather exceptional inasmuch as a dressing of lime in some form is absolutely essential to obtain a reasonable crop. At the same time, the responses to treatment shown by plants are very much greater than those shown by the fungus.

With respect to soil B, the crop yield is not influenced by changing the soil reaction and the weights of mycelium obtained with the cropped soils are not affected by the treatments. The cropping has effected a large decrease in available potassium, however, and a significant decrease in available phosphate in the limed soil. In the uncropped soils, liming has increased the available potassium and sulphur has decreased the available phosphate. A series of samples of soil B from a field experiment, carried out on the same lines as the above pot experiment, were examined by the A. niger method. The pH values of the different plots varied from 4.9 to 7.2 but the weights of mycelium did not vary beyond experimental error.

The data are not sufficient to justify any generalisation but suggest that the effect of the addition of calcium on the growth of Aspergillus is more closely related to the lime status and degree of saturation of the soil than to the presence of free calcium carbonate. In any case, it would seem that the normal applications of lime added to correct soil acidity would not influence the development of the fungus to any extent.

8. COMPARISON WITH MITSCHERLICH METHOD./

8. COMPARISON WITH MITSCHERLICH METHOD.

Through the courtest of Professor Mitscherlich, forty samples of soil, which had been examined by his well known culture method, were obtained for examination by the Aspergillus method. The results have been plotted against the corresponding Mitscherlich figures and the regression lines inserted (Fig. 1). The values for the potassium estimations are rather scattered, but it is worth noting that four of the five soils, represented by the encircled points above the line, have a high apparent density, and that the two marked below the line have a low apparent density. The point marked in the phosphate graph also represents a soil of high apparent density. It is not suggested that this explains all the discrepancies, for several exceptions could also be pointed out, but, since it covers the majority of the most discordant cases, it directs attention to the desirability of taking apparent density into consideration in the comparison of different soils, especially when the content of organic matter becomes high.

The correlation coefficients were calculated from the formula $r = \frac{\Sigma (m \times a)}{(n-1)\sigma_m \sigma_a}$ (18), where m and a represent the respective deviations from their means of the Mitscherlich and Aspergillus results; σ_m and σ_a are the standard deviations in the two cases, and n is the number of comparisons.

In the estimation of available phosphate, $r = 0.77$ which is quite significant for 40 observations; $r = 0.40$ in the case of available potassium and is also definitely significant, the probability that the agreement would be exceeded by chance being just greater than .01.

These results are quite different from those of Niklas, Miller and Frey (6). These authors obtained correlations of a very high order between the Aspergillus and the plant culture methods/

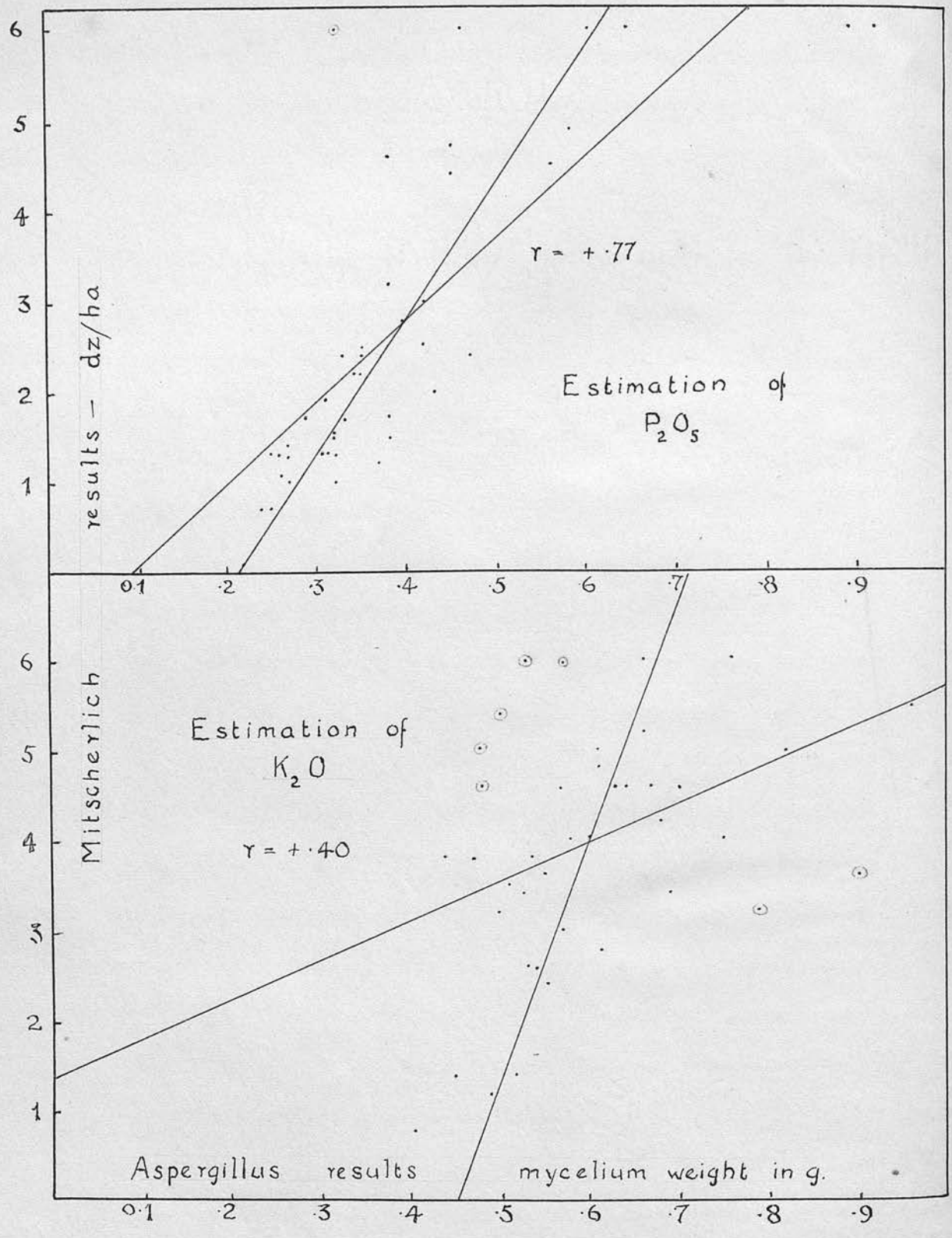

Fig. 1. Comparison of Aspergillus and Mitscherlich results: 40 Samples.

methods in the estimation of potassium, but found that the Aspergillus method agreed better with the chemical extraction methods in the estimation of phosphate. Good agreement between the Aspergillus and acid extraction methods is to be expected (13) since the growth of the fungus depends upon the quantities of potassium or phosphate absorbed from the soil, and these in turn are largely determined by the citric acid in the nutrient solution. As discussed at length by Reuter (11), the availability of soil phosphorus to A. niger also depends upon the source of carbon in the nutrient solution. Agreement between two methods, which differ fundamentally, is much more promising and the large grouping of low values for phosphate in Fig. 1 is very satisfactory from the point of view of advisory work. On the other hand, the majority of the soils might be described as moderately well supplied with available potassium, and it is with such a group that the greatest discrepancies between any two methods are likely to occur. It is also with such samples, unfortunately, that reliable information is most required, so that the relative lack of agreement between the two methods is somewhat disappointing.

It is useless discussing the discrepancies between the two methods, however, without information on the behaviour of the soils under field conditions and the above comparison was made simply because the Mitscherlich figures provided the best available alternative.

9. DISCUSSION OF RESULTS.

One striking feature of the results reported by many investigators, and which is also illustrated in Fig. 1, is that the Aspergillus method is not nearly so sensitive as the other common methods of examining soils. The range of Mitscherlich values, for example, is two or three times as great as that of the Aspergillus results for the same soils. In another set of soils the figures/

the figures for exchangeable potassium lay between 4 and 20 whilst those for mycelium weight lay between 0.2 and 0.5. The same thing is to be observed in the comparison of Aspergillus reaults with those obtained by the Neubauer or extraction methods (5, 9). One reason may be that the mycelium weight is not a direct measure of the nutrient uptake, for, as Vilsmeier has shown (21), the composition of the mycelium varies irregularly. According to the results of Mehlich, Truog and Fred (5), the nutrient absorbed by the fungus really agrees more closely than mycelium weight with the results obtained by other methods, but the necessity for analysing the mycelium would make the method impracticable. A few interpolation curves like that prepared by the above investigators (5) to give the relationship between the weight and composition of the mycelium would be extremely valuable.

It seems to be reasonable to assume from the data available that, whatever inconsistencies may be observed in the behaviour of the organism in presence of different types of soil, the decisive factor in the growth of the organism is the nutrient content of the soil. Probably the most serious criticism which can be directed against the method is that the end point is taken to be the same for different soils in spite of the individual factors which must influence the speed of growth and prevent the selection of the point of maximum yield. The behaviour of the fungus, however, seems to be sufficiently constant for the purpose of placing soils into two or three large groups, according to their fertiliser requirement, within which small differences in the weight and composition of the mycelium are of little consequence. There remains the difficulty of assessing borderline cases, but that is common to all methods of estimating soil fertility. The cheapness and speed of the method are great advantages, but a much greater variety of soil types from suitable

field/

field experiments must be examined to establish its reliability.

10. SUMMARY.

An examination has been made of certain factors which affect the development of A. niger in soil investigations. It is shown that considerable latitude is permissible in the concentration of inoculum employed, without sacrifice of accuracy; that ammonium nitrate or sulphate is better than ammonium citrate as a source of nitrogen; that due attention must be given to the area of cross section of the vessels employed.

A study has been made of the variability of the results from seven soils and three strains of the organism. It is shown that strain exerts a specific effect in the estimation of potassium, and that there is a significant interaction between soil and strain in the estimation of potassium or phosphate. The accuracy of the method is represented by a percentage standard error of about 2.3 for the mean of quadruplicate observations.

A few results have been given to show that normal dressings of lime to a soil are unlikely to affect the development of the organism.

A comparison of the Mitscherlich and Aspergillus results for forty soils has been made. The correlation coefficient is high (.77) in the case of the phosphate figures and lower but quite significant (.40) in the case of the potassium figures.

The general relationship between the Aspergillus and other methods is discussed and attention drawn to the necessity for testing the reliability of laboratory methods by field experiment.

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APPENDIX X

Estimation of available potassium by A. niger.

Analysis of variance of variance :-

	Sum of Squares	Degrees of Freedom	Mean Square
Strains	48,350	2	24,175
Soils	727,737	6	121,290
Remainder	787,449	12	65,621
Total	1,563,536	20	78,177 =

variance of variance based on 20 degrees of freedom.

Assuming that the variation in variance is due to random errors, the theoretical value of the variance of variance should be

$2 \times (\text{mean variance})^2 \div 10 = 2 \times 322.3^2 \div 10$

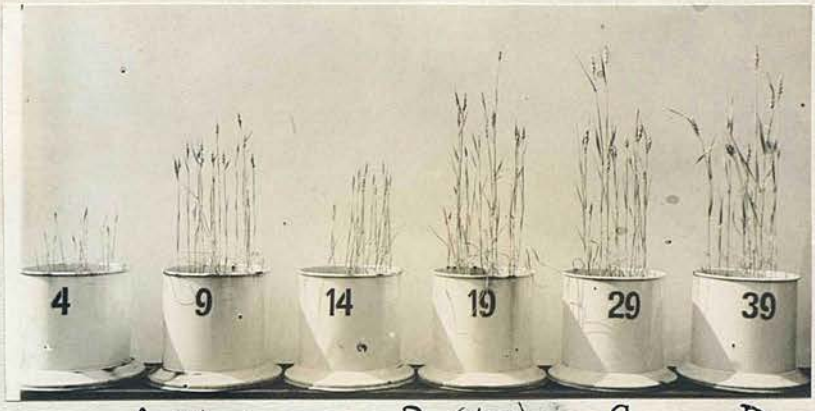
$= 20,776$

based on $9 \times 21 = 189$ degrees of freedom

$z = \frac{1}{2} \log_e 3.76 = 0.6625$ which is quite significant, i.e. the variability is not homogeneous.

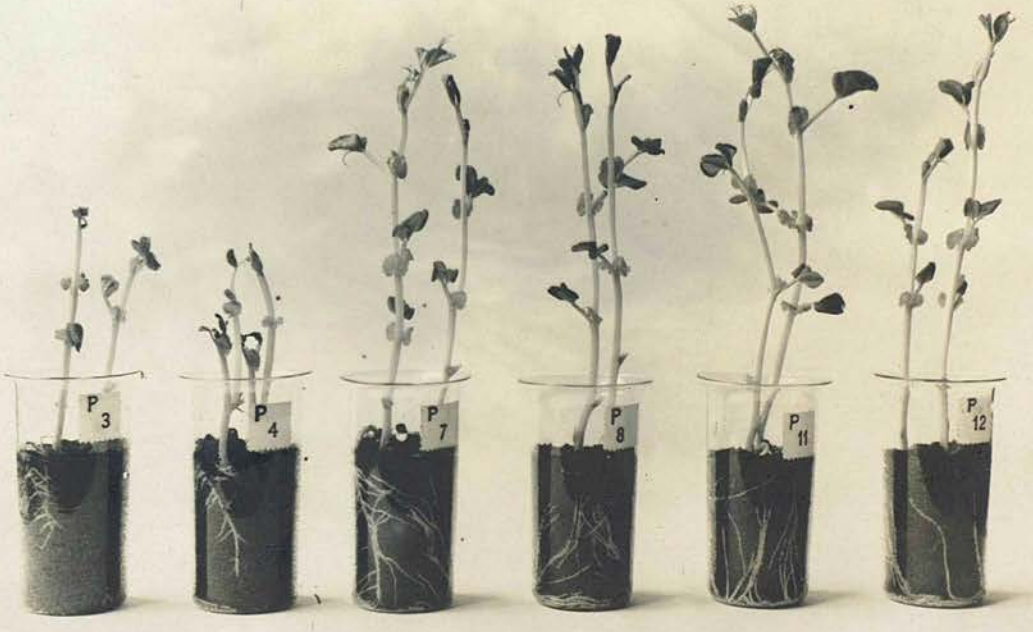
No 1. B2 B3 B4

Soil B (2·8·33)
see fig. 6a.



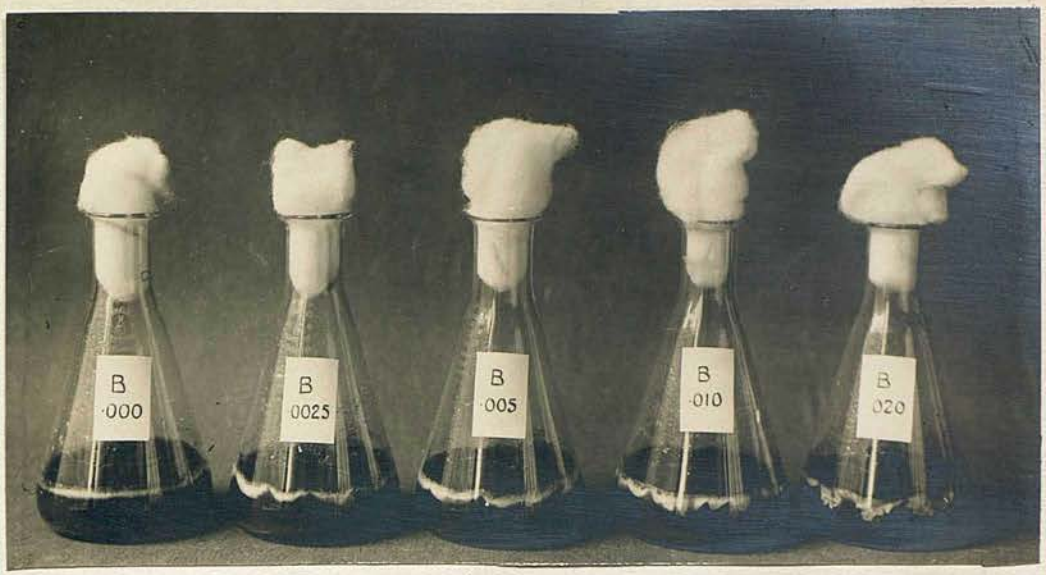
No. 2. A (slag) B (slag) C D

Soil W (2·8·33)
see fig. 7.



No 3. Soil P P + 1% $Ca(OH)_2$ P + 2% $Ca(OH)_2$

Soil P. see fig. 11.



No. 4.

The use of flasks and bottles in the A. niger method. (See p. 143)

No 5.

Copies of papers 3, 4, 5, 6, 7, 8, 9, 12.

[From THE ANNALS OF APPLIED BIOLOGY, Vol. XVI, No. 2, May, 1929.]

INVESTIGATIONS ON *HETERODERA SCHACHTII* IN LANCASHIRE AND CHESHIRE

PART II. THE RELATIONSHIPS BETWEEN DEGREE OF INFESTATION AND HYGROSCOPIC MOISTURE, LOSS ON IGNITION AND *p*H VALUE OF THE SOIL

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(With 2 Text-figures.)

It has been shown (Part I) that an infestation of *Heterodera schachtii* may not be uniform over even a small area. It seemed desirable, therefore, to make an examination of certain soil characteristics to see if any relationship existed between the degree of infestation and the nature of the habitat. It was thought that the rate of reproduction of the nematode might be influenced by moisture and temperature conditions. Hence, attention was first directed to those physico-chemical properties which are directly related to soil moisture and temperature.

Moisture and Loss on Ignition.

Keen and Russell (3) have produced data which show qualitatively that the moisture variations in a soil are inversely related to the mean temperature; in other words, the soil warms as it dries and *vice versa*, and a rapid rise of temperature, in spring for example, does not occur until the soil has lost its excess of moisture. In another investigation on single value determinations, Keen and Coutts (4) have shown that a good correlation exists on the one hand between content of organic matter and the moisture at the "sticky point," and on the other hand, between clay content and the equilibrium moisture content at 50 per cent. humidity. The last mentioned is in turn closely related to "air dry moisture." The soils in the present investigation were either peaty sands or peats and it seemed, therefore, that the simplest method of obtaining comparative figures for field conditions of moisture and temperature was to determine the moisture content and the loss on ignition of the air-dried soil.

All the samples concerned were made in the course of a few days,

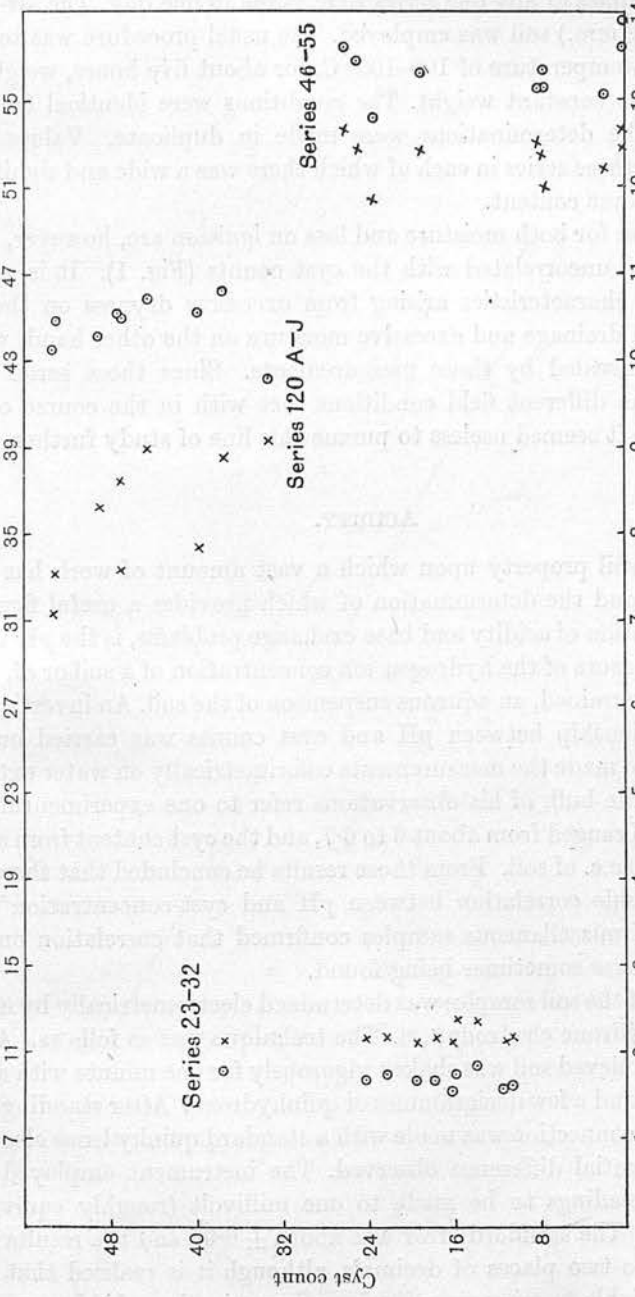

Fig. 1. Diagram showing relationship between infestation of *H. schachtii* and moisture, and loss on ignition for three series of soil samples.

while the samples of any one series were taken in one day. The air-dried and sieved (2 mm.) soil was employed. The usual procedure was to heat the soil to a temperature of 100–105° C. for about five hours, weigh and then ignite to constant weight. The conditions were identical for each series and the determinations were made in duplicate. Values were obtained for three series in each of which there was a wide and significant variation in cyst content.

The values for both moisture and loss on ignition are, however, quite scattered and uncorrelated with the cyst counts (Fig. 1). It is almost certain that characteristics arising from excessive dryness on the one hand, or bad drainage and excessive moisture on the other hand, would have been revealed by those measurements. Since those series were typical of the different field conditions met with in the course of the investigation it seemed useless to pursue this line of study further.

Acidity.

Another soil property upon which a vast amount of work has been carried out, and the determination of which provides a useful figure in the consideration of acidity and base exchange problems, is the *p*H value. It gives a measure of the hydrogen ion concentration of a soil or of, what is usually determined, an aqueous suspension of the soil. An investigation on the relationship between *p*H and cyst counts was carried out by Peters (5), who made the measurements colorimetrically on water extracts of the soil. The bulk of his observations refer to one experimental field where the *p*H ranged from about 6 to 6·7, and the cyst content from about 3 to 80 per 10 c.c. of soil. From those results he concluded that there was "an indubitable correlation between *p*H and cyst-concentration" but that a set of miscellaneous samples confirmed that correlation only in part, the reverse sometimes being found.

The *p*H of the soil samples was determined electrometrically by means of the quinhydrone electrode (1, 2). The technique was as follows. About 10 gm. of the sieved soil was shaken vigorously for one minute with about 25 c.c. water and a few decigrammes of quinhydrone. After standing for a few minutes, connection was made with a standard quinhydrone electrode and the potential difference observed. The instrument employed permitted the readings to be made to one millivolt (roughly equivalent to 0·02 *p*H). The standard error was about $\pm$ 0·02 and the results have been given to two places of decimals, although it is realised that such accuracy is liable to misconception in an investigation of this nature.

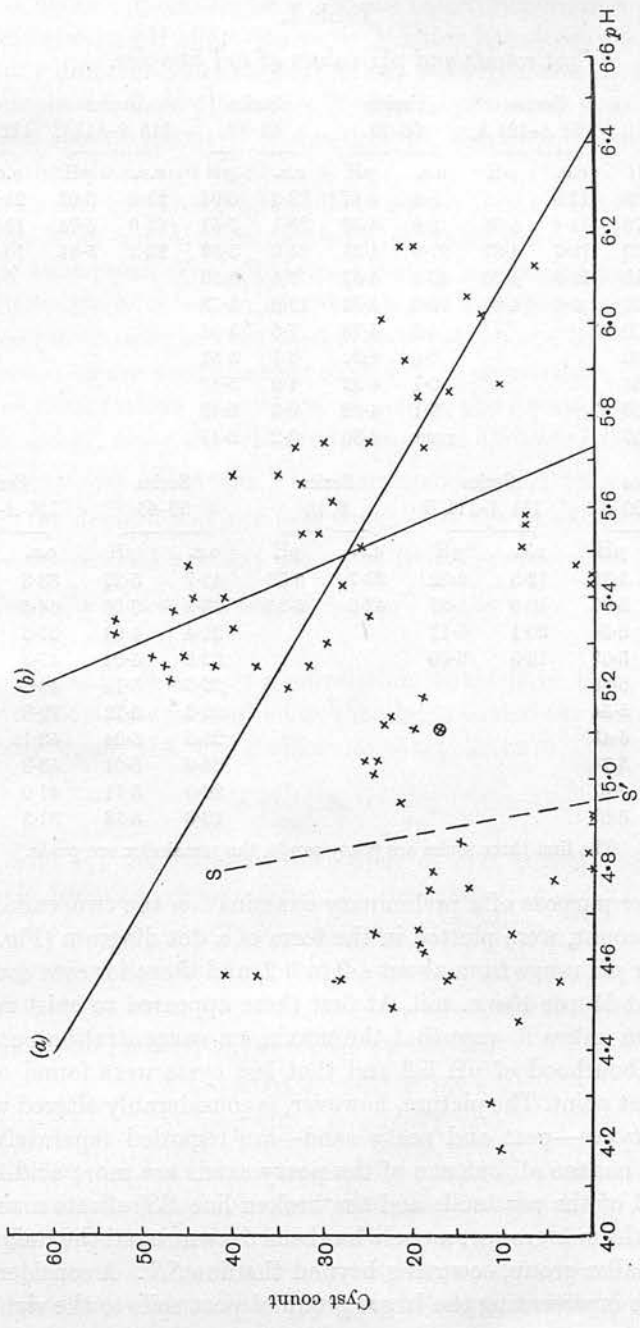

Fig. 2. Diagram showing association between cyst count and *p*H of soils.

Table I.

Cyst counts and pH values of soil samples.

Series 23–32		Series 124 *A*–124 *E*		Series 66–75		Series 46–55		Series 113 *A*–113 *C*		Series 112 *A*–112 *D*	
c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H
16·3	4·56	17·0	5·11	8·4	4·47	23·5	6·01	21·0	5·92	21·7	6·17
14·0	4·76	14·8	4·86	3·8	4·56	26·1	5·51	25·0	5·74	19·7	5·84
11·4	4·29	19·7	4·67	27·9	4·56	24·9	5·36	29·2	5·61	16·0	5·85
10·6	4·19	18·6	4·76	40·8	4·57	7·4	5·56			6·6	6·13
15·9	4·57	9·0	4·66	19·0	4·62	19·0	5·73				
22·3	4·50			4·3	4·78	7·6	5·58				
17·8	4·80			0·6	4·84	8·1	5·51				
19·5	4·64			0·1	4·92	1·9	5·47				
24·3	4·66			0·1	4·92	0·3	5·43				
37·5	4·57			none	4·80	0·2	5·45				

Series 11–20		Series 111 *A*–111 *D*		Series 6, 10		Series 56–65		Series 120 *A*–120 *J*	
c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H
46·7	5·37	12·5	6·02	29·7	5·30	45·7	5·27	33·3	5·73
41·2	5·40	10·6	5·87	42·0	5·25	33·8	5·20	44·5	5·40
30·5	5·54	20·1	6·17			21·4	4·95	37·5	5·25
35·0	5·67	13·9	6·06			23·9	5·04	47·3	5·25
32·4	5·65					22·5	5·14	49·1	5·27
32·3	5·54					23·3	5·12	39·8	5·33
27·9	5·43					25·5	5·04	53·1	5·35
30·1	5·74					24·6	5·01	53·3	5·32
44·9	5·47					20·0	5·11	47·0	5·22
40·2	5·67					18·8	5·18	31·3	5·25

The first three series are peaty sands, the remainder are peats.

For the purpose of a preliminary examination the two variables, *p*H and cyst count, were plotted in the form of a dot diagram (Fig. 2). The figures for *p*H range from about 4·2 to 6·2, and those for cyst count from 0 to about 53 per 10 c.c. soil. At first there appeared to exist no simple association unless it were that the maximum concentration occurred in the neighbourhood of *p*H 5·3 and that less cysts were found on either side of that point. The picture, however, is considerably altered when the two soil types—peat and peaty sand—are regarded separately. By a fortunate chance all but one of the peaty sands are more acid than the most acid of the peat soils and the broken line *SS′* effects a simple division of the two groups: a circle has been drawn round the unique point of the smaller group, occurring beyond the line *SS′*. A consideration of the points representing the larger group of peat soils to the right of *SS′*

shows that there appears to be a general trend indicating a negative correlation between *p*H and cyst count. Mention has already been made of the totally different characters of the two soil types, and the figures of Table II emphasise those differences in physical properties as exemplified by apparent specific gravity. It was decided, therefore, to analyse the results for the two groups separately.

It was further considered advisable to regard all the pairs of observations as one large sample from an area over which the occurrence of *H. schachtii* is widespread. The *p*H through a strip 100 yards long on a field usually fluctuates to a certain extent owing to a variety of causes, but the range is rather short and ten pairs of observations at most, too limited in this case to secure a satisfactory coefficient of correlation. Taking the 53 pairs of observations as a whole, therefore, the figures for *p*H vary from 4·95 to 6·17, those for cyst count from 0 to 53·3 per 10 c.c. soil.

If X = any cyst count and Y the corresponding *p*H value,

x = the deviation of any cyst count from the mean $\overline{X}$ (27·25),

y = the deviation of any *p*H value from the mean $\overline{Y}$ (5·50),

then r, the coefficient of correlation $= \Sigma xy/\sqrt{\Sigma x^2 \Sigma y^2}$.

In this case $r = -0{\cdot}504$.

The probability that such a correlation would have been obtained from any random comparison is less than 0·01, so that the correlation is undoubtedly significant. The coefficients of regression (6)

$$b_1 = r\frac{\sigma_x}{\sigma_y},\ b_2 = r\frac{\sigma_y}{\sigma_x},$$

where σ_x and σ_y, the standard deviations of cyst counts and *p*H, are respectively 13·86 and 0·2361, have been calculated:

$$b_1 = -29{\cdot}59,\quad b_2 = -0{\cdot}008592.$$

The regression equations are therefore $x = -29{\cdot}59\,y$,

$$y = -0{\cdot}00859\,x,$$

or replacing x by $(X - 27{\cdot}25)$ and y by $(Y - 5{\cdot}50)$

$$X = 190{\cdot}05 - 29{\cdot}59\,Y \quad (a),$$

$$Y = 5{\cdot}7342 - 0{\cdot}00859\,X \quad (b).$$

The dot diagram has been completed by inserting the lines representing the equations (*a*) and (*b*). An examination of the diagram reveals that the correlation coefficient is reduced by the group of values for the observations 58–65 (see p. 336, Part I) all below *p*H 5·20 and with comparatively low cyst counts. They appear to belong to another population.

There is little doubt, therefore, that some correlation, worthy of further investigation, exists between cyst count and *p*H, but that there are other factors at play.

When the twenty-five pairs of observations for the sandy soils are subjected to the same treatment, the coefficient of correlation is found to be − 0·176 which is certainly not significant.

Table II.

Apparent specific gravities of two soil types.

Type	Peaty sand		Peat soil				
Sample	Composite 124 *B*		111 *A*	112 *A*	113 *A*	120 *A*	120 *I*
Apparent spec. grav.	1·20	1·23	0·55	0·61	0·54	0·61	0·54

SUMMARY.

1. Soil samples taken for the purpose of measuring the infestation of *Heterodera schachtii* have been further examined for any association between cyst concentration and those physico-chemical properties relating to the moisture, temperature and acidity of the soil.

2. The hygroscopic moisture and loss on ignition have been determined for three typical series of ten samples having significant variations in cyst counts. There is no simple association of the observations within any of the series. It appears improbable, therefore, that the rate of reproduction of *Heterodera schachtii* is influenced to a marked degree by the normal variations in the physical condition of the soil.

3. The *p*H of seventy-eight samples has been measured electrometrically. When the samples are divided into two groups according to soil type, there is found to be a significant negative correlation between *p*H and cyst count for fifty-three peat soils. There is not, however, a significant correlation in the case of the other twenty-five sandy soils.

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INVESTIGATIONS ON *HETERODERA SCHACHTII*, SCHMIDT. IN LANCASHIRE AND CHESHIRE

PART III. CERTAIN CORRELATIONS BETWEEN CROP YIELDS AND DEGREE OF INFESTATION

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(With 1 Text-figure.)

In a previous publication (2) the technique of estimating the degree of infestation of *H. schachtii* in the field was examined and an attempt made to correlate significant differences with the intensity of "eelworm disease" as estimated by visual observation. Although the results indicated that in those areas where disease had been noted comparatively recently there was a positive association between cyst count and intensity of disease, it was felt that a more accurate computation of the latter, by measuring crop yield, was necessary before any definite conclusion might be drawn. Furthermore, the fact that, in areas where disease had been observed more than three or four years previously, the cyst counts did not bear a close relationship to the state of the crop, indicated that *H. schachtii* was at most only one factor in the causation of what is termed "eelworm disease" of potatoes. It was decided, therefore, to lay down a number of plots on affected ground, make observations throughout the growing season, weigh the produce and compare the yields with the original and final degree of infestation as measured by cyst counts.

Experimental.

Field. Four series of plots, varying in size from 1/130 to 1/40 of an acre were laid down in different localities. The first three series were on peat and the fourth on peaty sand. The soil characteristics and farm practice, together with the notes on the first appearance of disease, have

already been fully described (2). The plots were sampled about the middle of January, 1928, and measurements made of cyst content and a number of chemical properties. About the middle of March, 16 of the 29 plots were treated with varying amounts of calcium carbonate to provide data for another object in view. All the plots received the same manurial dressing in May, prior to planting with potatoes. The variety employed was Great Scot and all the seed was taken from one consignment. Field notes were made throughout the growing season and the crops were harvested at the beginning of September, the weights being taken to the nearest pound. The plots were re-sampled in December and the same measurements made as on those samples of soil which were taken nearly twelve months previously.

Laboratory. The soil samples were allowed to reach an air-dry condition and then passed through a 2 mm. sieve. Cyst counts were made as described (*loc. cit.* p. 326) on the fine earth portion. For each sample determinations were made of (*a*) the *p*H electrometrically (3), (*b*) the "lime-requirement" by the Hutchinson and MacLennan method, and (*c*) the content of free carbonate by means of a Collin's calcimeter. The results have been collected in Table I.

Results.

Field observations. So far as could be gathered from observations made in the field, growth commenced quite normally, and little or no difference could be noted on the different plots. There was, however, a considerable number of misses associated directly with the fungus *Rhizoctonia solani* Kühn. By the middle of August it was quite obvious that all the crops were far below the average and that, in the series 1 and 3, they were, from the practical point of view, almost complete failures. In those cases the foliage had an unhealthy appearance and was so dwarfed that the drills were not completely covered. Series 2 was not quite so bad, but many poor patches were to be seen at irregular intervals. Series 4 was undoubtedly the best, but even there the crop was not up to standard.

Laboratory measurements. In Table I the limed and unlimed plots have been kept separate in order to facilitate comparison. Figures for the total yield of potatoes, including ware and chats, are given alongside the original, final and change in cyst count. The difference between the amount of calcium carbonate added and the original "lime-requirement" was closely associated with the final content of carbonate in the soil: since, however, the unlimed plots almost invariably showed an increase

Table I.

Results for series of plots.

Unlimed.						Limed.					
	Cyst count per 10 c.c. soil						Cyst count per 10 c.c. soil				
No.	Before	After	Change	Yield cwt./A	% $CaCO_3$ Change	No.	Before	After	Change	Yield cwt./A	% $CaCO_3$ Change
1 AB	11·8	11·1	−0·7	36	Nil	1 A	12·5	11·7	−0·8	40	2·75
1 BC	14·1	12·7	−1·4	23	0·15	1 B	10·8	13·0	+2·2	30	1·36
1 CD	17·8	12·5	−5·3	26	0·18	1 C	18·8	9·8	−9·0	19	1·99
1 DE	11·5	14·9	+3·4	39	Nil	1 D	14·7	15·0	+0·3	30	1·22
2 AB	20·8	22·7	+1·9	76	0·10	2 A	21·7	26·8	+5·1	114	0·35
2 BC	17·8	27·4	+9·6	94	0·29	2 B	19·7	25·6	+5·9	60	2·48
2 CD	10·6	15·5	+4·9	57	0·05	2 C	16·0	20·3	+4·3	53	0·93
						2 D	6·6	12·1	+5·5	105	0·87
3 A 1	21·0	13·3	−7·7	26	0·76	3 A 2	21·0	11·5	−9·5	34	1·52
3 B 2	25·0	21·8	−3·2	14	0·04	3 B 1	25·0	19·9	−5·1	28	1·24
4 A 3	17·0	20·7	+3·7	134	0·04	4 A 1	17·0	16·4	−0·6	122	0·72
						4 B 1	14·8	19·5	+4·7	119	0·28
4 C 1	19·7	23·9	+4·2	117	0·01	4 C 2	19·7	18·4	−1·3	91	0·14
4 D 3	18·6	19·4	+0·8	86	0·11	4 D 2	18·6	17·7	−0·9	87	0·30
4 E 2	9·0	12·8	+3·8	81	0·07	4 E 3	9·0	11·9	+2·9	108	0·25
						4 B 2	14·8	18·2	+3·4	99	1·26

in content of carbonate, owing, presumably, to the accidental transfer of lime from the adjacent limed plots, the increase in percentage of calcium carbonate has been given throughout. In view of the heavy dressings of lime and the comparatively short space of time elapsing since their application, not much stress could be laid upon the final *p*H figures for the limed plots, whilst the *p*H of the unlimed plots did not alter materially. Consequently, the *p*H values have not been included.

The most striking feature of the results is the very low yields obtained from the diseased areas upon which the plots were laid. A normal yield fluctuates about 10 tons per acre, but on those plots the yields vary from less than 1 ton to about 6 or 7 tons per acre; the average yield is little more than 30 per cent. of the normal.

As a preliminary step towards the elucidation of any associations, scatter diagrams were prepared from the various pairs of values. It was evident that no close association existed between the yield and the amount of calcium carbonate present or between the yield and the original cyst concentration of the soil. There was an apparent negative association of values representing change in cyst count and original cyst count, and the same thing could be said to a less degree of the change in cyst count and the increase in calcium carbonate. The most obvious association, however, lay between yield of potatoes and change in cyst

count. An examination of the data shows that, with few exceptions, the cyst count has increased when the yield is more than 50 cwt. and decreased when the yield is less than 50 cwt. The figures for yield and change in cyst count are plotted in Fig. 1, and the general trend of the results is fairly obvious.

In order to examine the possible associations more closely, "total correlation" coefficients for six pairs of values have been calculated and are shown in Table II.

Table II.

Coefficients of correlation between pairs of values.

	A	*O*	*L*
Y	+0·592	−0·131	−0·315
A	—	−0·409	−0·232
O	—	—	+0·044

The letters *A*, *O*, *L*, *Y* designate, respectively, alteration in cyst count, original cyst count, increase in percentage of calcium carbonate and yield. The correlation between *A* and *Y* has been calculated from the formula (1),

$$r_{AY} = \frac{\Sigma ay}{n\sigma_a\sigma_y},$$

where $\Sigma ay/n$ = the mean product of the deviations of *A* and *Y* from their means,

σ_a = the standard deviation of *A*,

σ_y = the standard deviation of *Y*.

For 29 sets of observations, the only correlation which is definitely significant is that between *A* and *Y*. The coefficient $r_{AO} = -0{\cdot}409$ has a probability between 0·05 and 0·02 and is, therefore, just significant.

The correlations between *A* and *Y* when *O* and *L* are eliminated are

$$r_{AY.O} = 0{\cdot}596 \quad \text{and} \quad r_{AY.L} = 0{\cdot}562,$$

which show that the association between *A* and *Y* is independent of the original cyst concentration, and is reduced slightly by the elimination of *L*, the increase in calcium carbonate present. It is not possible to say whether *A* and *Y* vary independently or the extent to which they may be interdependent, but certain factors apparently affect *A* and *Y* similarly and to an important extent compared with other factors, so that, generally speaking, over the diseased areas investigated the cyst concentration increases in proportion to the yield when that is greater than about 3 tons per acre, and decreases with yield when that is less than about 3 tons per acre.

The regression lines (*a*) and (*b*), represented by the equations

$$A = 0{\cdot}0737\,Y - 4{\cdot}215 \qquad \ldots(a),$$

$$Y = 4{\cdot}75\,A + 63{\cdot}73 \qquad \ldots(b),$$

have been inserted in Fig. 1. Those equations have been obtained from the coefficients of regression $b_1 = r\sigma_a/\sigma_y$ and $b_2 = r\sigma_y/\sigma_a$, from which $a = 0{\cdot}0737\,y$ and $y = 4{\cdot}75\,a$. The mean values for A and Y are respectively 0·73 and 67·2, so that $a = (A - 0{\cdot}73)$ and $y = (Y - 67{\cdot}2)$.

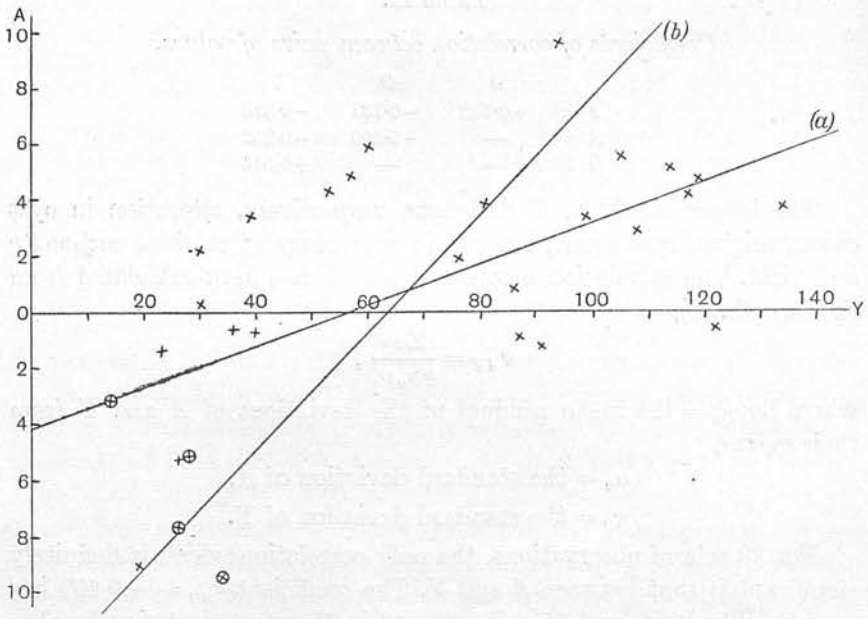

Fig. 1. Diagram showing association between yield (Y) and change in cyst content (A).

It is evident that the correlation depends upon a number of low values for Y, four of which are from series 3 and have been encircled. On account of the limitations of the experiments, a close analysis of the plot variation cannot be made, but it is possible that the association between A and Y may exist in the variations between plots and be bound up with the unknown factor fertility.

The coefficient of correlation $r_{AO} = -0{\cdot}409$ is not affected much by eliminating L and Y because $r_{AO.L} = -0{\cdot}411$ and $r_{AO.Y} = -0{\cdot}414$. The data, however, do not reveal whether the association between the original concentration and change in concentration of cysts is due to a causal relationship or whether both are affected by another factor. It

has been shown (3), for the soils under consideration, that no apparent association exists between cyst count and physical environment. The observed correlation between A and O simply indicates that, under the conditions of the investigation, the number of cysts tends to increase on cropping when the original concentration is less than about 17 and *vice versa*. It must be noted, however, that without the four large negative values for A from series 3 the observed correlation would become negligible.

Summary.

1. Data concerning infestation of *H. schachtii*, certain soil properties and crop yield, from a series of plots at four centres affected with disease, have been collected and examined.

2. Limitations imposed on the design of the plots have prevented a complete analysis of the variates, but the results obtained lead to the conclusion that eelworm infestation is not of primary importance in determining the yield of potatoes and that the cysts tend to increase in number only on a crop which is not a failure but which has been adversely influenced by some other factor.

The authors would like to express their thanks to Messrs I. S. Macdonald, J. Orr, E. Holmes Smith and I. Thomas of the Advisory Department at Manchester for their willing co-operation and assistance, and to Mr L. H. C. Tippett for his helpful criticism of the statistical treatment of the results.

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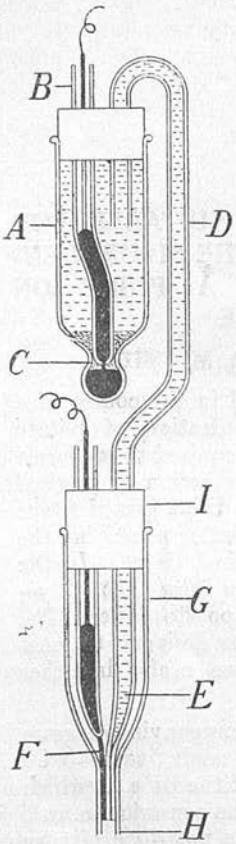
A CAPILLARY ELECTRODE SUITABLE FOR THE DETERMINATION OF THE HYDROGEN-ION CONCENTRATION AT A POINT ON PLANT TISSUE

By I. M. ROBERTSON and A. M. SMITH

Two difficulties were encountered in the course of a study of the hydrogen-ion concentration of potato tubers. In the first place, the time required to prepare a pulp was sufficient to allow enzyme action to proceed to a considerable distance, and the disintegrated tissue could not with confidence be regarded as possessing the same degree of acidity as the original tissue. In the second place, there is no evidence to show that the p_H value is uniform throughout the potato tuber. The apparatus described in this note was designed to meet those difficulties, and it may prove useful in other investigations of a similar nature.

Description of apparatus.—The accompanying diagram is drawn to scale. The vessel A is a small " saturated " calomel half-cell, the rounded end of the tube B, which carries the connecting wire, fitting the constriction at C fairly closely so that the mercury is not disturbed by tilting. D is a saturated potassium chloride–agar bridge ending in a fine capillary at E. This capillary is sufficiently long to permit of the periodic removal of small pieces from the end, so as to expose a fresh surface of

potassium chloride–agar. The platinum wire F, beaten out at the end, lies close to and protects the point E. It is kept in position by the tube G, which is drawn out at H so that the internal diameter is less than 1 mm. The ends of the wire F and the capillary E reach a point about 2 mm. from the end of the tube G. The whole apparatus is only 14 cm. long and weighs about 40 g., so that it can be held like a pencil.



The procedure adopted was to place a few crystals of quinhydrone on the tissue at the point under investigation and then insert the point H. The plant juice, together with some quinhydrone, rose in the capillary to meet E and F and the potential difference was determined by means of a potentiometer circuit, the accuracy of which was about 0·5 millivolt.

To clean the apparatus for another determination, the tube G was slipped off the rubber stopper I and a jet of water directed where necessary. In addition, the stopper was partially split so that the tube carrying F could be removed easily to permit of the wire being flamed without endangering the capillary E.

Statement of results.—A comparison of values obtained for our buffer solutions by this capillary electrode and

by means of an ordinary quinhydrone–calomel system (Biilmann and Lund, Ann. Chim., 1921, **16**, 321 *et seq.*) is presented in Table I. Each figure represents the average of almost identical replicates. The capillary electrode was quite as delicate as the other and yielded results which were essentially the same.

TABLE I

p_H Values of certain buffer solutions

Capillary electrode	Ordinary electrode
3·98	3·99
4·92	4·92
6·07	6·06
7·09	7·07

A large number of tubers were examined for variation of p_H value from point to point on the tissue. Determinations were usually made at about 12 points on a plane through the " heel " and " rose " ends of the tubers, and an average variation of 0·08 p_H unit was found. For the present purpose average values have been taken.

Groups of five tubers were examined by this method and the average p_H value of the tissue compared with that of the pulp prepared from the same groups of tubers. A few results are given in Table II, which illustrate typical differences obtained.

TABLE II

Average p_H of tissue obtained by capillary electrode compared with the p_H of pulp

Tissue	Pulp
5·86	5·76
5·70	5·76
5·68	5·74
5·67	5·71

It will be observed that the hydrogen-ion concentration of the pulp is always lower than that of the tissue, which may be accounted for as follows. The loss of carbon dioxide, present in the plant sap, during the process of pulping probably results in a decreased hydrogen-ion concentration. Furthermore, the oxidase activity in the disintegrated tissue is very great and, according to

Raper (Biochem. J., 1927, **21**, 89), brings about the conversion of tyrosine into indole derivatives which are intermediate products in the formation of melanin. It is not known exactly how those transition compounds would influence the hydrogen-ion concentration, but formation of the final insoluble product, melanin, would doubtless reduce the acidity.

It is probable, however, that loss of carbon dioxide is the more potent factor, since determinations of the p_H of the pulp by means of a hydrogen electrode gives results which are even higher than those obtained by the ordinary quinhydrone electrode.

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LXXXV. A STUDY OF THE HYDROGEN ION CONCENTRATION OF THE POTATO TUBER.

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(*Received April 3rd, 1931.*)

Numerous investigations have been made on the acidity of plant sap and several methods have been employed. The hydrogen electrode has been used extensively but the time required for the system to reach equilibrium, the alteration of the biological fluid by the passage of hydrogen and the poisoning of the electrode by the plant products are obvious disadvantages of the method. Colorimetric methods are not entirely satisfactory on account of the rapid colour changes which may take place in the sap as a result of enzyme action. With the quinhydrone electrode, the precision of which has been critically examined by Morgan, Lammert and Campbell [1931], equilibrium is attained very rapidly and manipulation is simple. Plant oxidases, however, attack quinol [Chodat, 1910] and, consequently, the ratio quinol : quinone is liable to be altered. In this paper, the results obtained by rapid measurement of the acidity of tuber tissue with a special quinhydrone capillary electrode are described and compared with those obtained by other methods.

Methods.

(*a*) *Extraction of sap.* When it was desired to examine the sap, the tubers were washed in cold water and passed through a pulping machine. The mash was filtered through fine linen and the filtrate, which gave essentially the same results as the pulp, was generally employed. The pulped tubers decomposed rapidly so that the sap usually had a red-brown colour which became darker on standing.

(*b*) *Hydrogen electrode measurements.* A Hildebrand half-cell was used in conjunction with a saturated calomel electrode. About 5 cc. of the plant sap were taken for each determination and equilibrium was reached after approximately 10 minutes. The hydrogen electrode was given a fresh deposit of platinum black after each measurement.

(*c*) *Quinhydrone electrode measurements.* The quinhydrone electrode consisted of a piece of polished platinum foil which was placed in the potato sap saturated with quinhydrone. Some preliminary experiments showed that at least 0·12 g. of quinhydrone had to be added to each 10 cc. of sap in order to

obtain reproducible results. The procedure adopted was to shake the sap with sufficient quinhydrone for a few seconds, complete the potentiometer circuit with the platinum and saturated calomel electrodes and read the E.M.F. of the cell at once. The whole operation was carried out in less than 1 minute but decomposition of the phenol had already commenced. The platinum foil was washed and heated to redness in an alcohol flame after each measurement and from time to time was also cleaned in hot chromic acid solution.

A comparison of results obtained by methods (*b*) and (*c*), included in Table III, shows that in most cases the hydrogen electrode yields slightly higher p_H values. This may be due to some alteration in the sap during the passage of hydrogen or to some error in the application of the quinhydrone half-cell. The fact that the sap, if allowed to stand for about 10 minutes before the addition of the quinhydrone, gave approximately the same value as with the hydrogen electrode, suggests that the time required by the system to reach equilibrium is the important factor.

(*d*) *Micro-electrode measurements.* Reproducible results were readily obtained with the quinhydrone electrode, but the use of pulp in any method removes the possibility of studying the variation in acidity throughout the tuber. To meet this difficulty a special electrode was constructed [Robertson and Smith, 1930], which enabled measurements to be made at any desired point on a section of the plant tissue. A few results obtained by means of this electrode and by the method described under (*c*) are given in Table IV. The p_H of the sap was always greater than that observed with the micro-electrode for the tissue, which was probably due to loss of carbon dioxide in the preparation of the pulp. Ingold [1929] observed a reduction of 0·3 p_H unit when the tuber sap was brought into equilibrium with concentrations of carbon dioxide such as have been recorded for the intercellular spaces of the tuber. In addition, the oxidase activity of the disintegrated tissue is very great and the formation of melanin and its intermediate compounds from tyrosine [Raper, 1928] may also reduce the acidity.

Variation in acidity throughout the tuber.

Although some data are available on the hydrogen ion concentration gradients of the sap of plants [Gustafson, 1924], no such observations seem to have been made on the potato tuber. Attention was directed to a study of the changes which take place during the formation, storage and sprouting of the tuber.

(*a*) *During growth period.* A number of healthy and typical plants was selected and examined at intervals throughout the summer. The tubers were cut along a plane through an eye and the heel. Readings were taken with the micro-electrode at points in the middle and 1–2 mm. below the eye and heel. The results for two varieties given in Table I are typical of a large number of observations and each figure is an average value for four tubers.

Table I. *Variation of tuber* p_H *with stage of growth.*

Variety	Date of sampling ...	30. vi. 30	21. vii. 30	16. viii. 30	30. ix. 30
Duke of York	"Rose" end	6·19	6·04	5·90	5·65
	Centre	6·20	6·08	5·75	5·71
	"Heel" end	5·53	5·90	5·85	5·73
Epicure	"Rose" end	6·08	6·13	5·81	5·68
	Centre	6·06	6·09	5·88	5·75
	"Heel" end	5·78	5·82	5·80	5·80

The first readings were made with tubers weighing 5–15 g. and it was observed that the underground stem attaching the tuber to the parent plant had approximately the same p_H as the heel of the tuber. The final sampling was made after the plants were completely ripe, the haulms having withered away some time previously. The results show that all parts of the tuber, except the heel, become more acid as the plant ripens. The p_H of the heel rises quickly and then falls off gradually. There is a p_H gradient across the young tuber, the heel being more acid than the rose end. In the mature tuber the reverse holds good. This change coincides with the physiological development of the tuber, for in the early stages of growth the active part is at the heel where nutrients are received from the plant, whereas in the mature state this process has ceased and the eyes develop rapidly.

(*b*) *During rest period.* A large number of tubers was examined between the time of harvesting and the appearance of sprouts. Observations were made at eleven points on a section of each tuber, both cortex and medulla being represented. In the majority of cases the rose end was slightly more acid than the heel end, the centre being intermediate. The values given in Table I for the last sampling are typical in this respect. In many tubers, however, no difference was noted between the rose and heel ends, while in a few isolated cases the heel was slightly more acid than the rose end. Generally, the average value for all the points approximated closely to that for the centre of the tuber.

(*c*) *During sprouting period.* In March a number of dormant tubers was exposed to daylight and allowed to sprout. At certain intervals a few tubers were cut down through a sprout and eye to the heel and the acidity was determined at a number of positions across the section. Tubers of nine varieties were examined and all yielded similar results. In Table II the average values for three tubers of the variety Duke of York are given.

Table II. *Variation of* p_H *during sprout formation.*

Position on tuber or sprout	20. iii. 30	16. iv. 30	15. v. 30	24. vi. 30
Growing tip of sprout	4·28	4·30	4·38	4·51
Middle of sprout	4·45	4·55	4·62	4·90
Tuber end of sprout	4·25	4·36	4·54	5·09
Surface of "eye"	4·58	4·73	5·70	5·85
2 mm. below surface of "eye"	5·31	5·60	5·86	—
5 mm. below surface of "eye"	5·61	5·67	5·83	—
10 mm. below surface of "eye"	5·75	5·74	5·86	—
Middle of tuber	5·74	5·71	5·78	5·80
"Heel" end of tuber	5·80	5·85	5·86	5·88

The following is a description of the sprouts:

20. iii. 30.—Sprouts soft and light green, and about 0·5 cm. long.
16. iv. 30.—Sprouts hard and dark green, 0·5–1·5 cm. long.
15. v. 30. —Sprouts thicker, 2·0–2·5 cm. long, small leaves forming.
24. vi. 30.—Sprouts very thick and hard, 3 cm. long, small leaves and shoots formed. Tuber soft.

When sprouts appear, the region round the active eyes is very acid, but gradually becomes less acid as the sprouting progresses until June, when the p_H is practically uniform throughout the tuber. The growing tip of the sprout retains its relatively high acidity throughout the growth of the plant; in a young field plant it had p_H 4·46 while in an older plant the p_H was 4·65.

These variations in p_H through the tuber are to be expected if consideration is given to the different functions of the various parts. The actively growing parts are usually more acid than the fully developed members. On the other hand, the acidity of the tuber increases with maturity, which may be accounted for by the fact that, according to Appleman and Miller [1926], the protein in the young tuber gives place to non-protein- and amino-nitrogen in the mature tuber.

Factors influencing the p_H *value of the mature tuber.*

(*a*) *Environment.* Tubers of six common varieties, grown at seven places in Midlothian, were examined immediately after harvesting. The altitudes of the different places varied from 100 to 600 feet above sea level; the different soils varied in texture from sandy loams to clay loams and in p_H from 4·9 to 7·3. There was no obvious relationship, however, between environment and the acidity of the tuber. The maximum variations of individual values for all sources were as follows: Duke of York, 5·70–5·89; Epicure, 5·70–5·85; Great Scot, 5·65–6·11; Golden Wonder, 5·70–5·85; Majestic, 5·50–5·73; Ally, 5·67–5·84.

In order to investigate more fully the effect of soil reaction upon the acidity of the tuber, a crop of variety King George, grown on a series of specially treated plots on a soil of p_H 4·0, was examined. The p_H of the tubers was measured by methods (*b*) and (*c*) and the results are summarised in Table III.

Table III. *Relation between* p_H *of soil and tuber sap.*

p_H of soil	No. of plots	p_H of tuber sap	
		Method (*b*)	Method (*c*)
4·0–4·5	4	5·69	5·66
4·5–5·0	11	5·68	5·64
5·0–5·5	11	5·66	5·63
5·5–6·0	5	5·69	5·63
6·0–6·5	3	5·68	5·63
6·5–7·0	2	5·62	5·68

In 1929, five plots were laid down on a soil having p_H 5·5. Two plots were made more acid by treatment with flowers of sulphur and two more alkaline by treatment with calcium hydroxide. Two varieties were grown on each plot.

In autumn, samples of tubers were lifted, washed and dried and then examined as follows. Ten tubers were selected at random from each sample and cut through the rose and heel ends. The acidity was determined at various points on the section by means of the micro-electrode. The small injuries made by the electrode were then cut out, the tubers were pulped and the p_H of the sap was determined by method (*c*). The average results are given in Table IV, the p_H values of the soil being those obtained during September.

Table IV. *Relation between* p_H *of soil and tuber.*

		Average p_H of tubers			
		Duke of York		Great Scot	
Plot	p_H of soil	(*d*)	(*c*)	(*d*)	(*c*)
A	8·1	5·70	5·77	5·68	5·73
B	7·5	5·68	5·75	5·69	5·73
C	5·5	5·70	5·74	5·69	5·70
D	5·1	5·70	5·73	5·68	5·74
E	5·0	5·71	5·77	5·68	5·72

It is obvious from the above results that the p_H of the tuber is not influenced by that of the soil. Hoagland and Davis [1925] have obtained similar results for other plants grown in culture solutions, but other workers, for example Haas [1920], have found a certain relationship between the acidities of soil and plant sap.

(*b*) *Variety.* In addition to the p_H figures already given for different varieties, the following were obtained: Arran Consul, 5·64; Field Marshal, 5·74; Kerr's Pink, 5·75; King Edward, 5·95. The differences between varieties were thus not significantly greater than the variations found among tubers of the same variety.

(*c*) *Disease.* A number of tubers affected by various diseases was collected and compared with normal tubers of the same variety. The influence of virus infection was also studied. The results are presented in Table V.

Table V. *The effect of disease upon the* p_H *of tubers.*

			Affected tuber	
Variety	Nature of infection	Normal tuber	Healthy part	Diseased part
Great Scot	Corky scab: *Spongospora subterranea* Lagerh.	5·70	5·65	4·35
Duke of York	Common scab: *Actinomyces scabies* (Thaxt.) Güssow	5·75	5·70	4·58
Epicure	Sprain: *Bacterium rubefaciens*	5·73	—	5·60
Epicure	Blackleg: *Bacillus atrosepticus* van Hall	5·73	5·68	5·65
Duke of York	Blight (fresh): *Phytophthora infestans* (Mont.) De By.	5·75	5·75	5·83
Duke of York	Blight (old)	5·75	5·70	5·38
Duke of York	Wart disease: *Synchytrium endobioticum* (Schilb.) Perceval	5·75	5·65	5·02
Ally	Mosaic	5·80	5·70	
	Crinkle	5·80	5·60	
	Leaf Roll	5·80	5·85	
Arran Comrade	Mosaic	5·64	5·47	
	Leaf Roll	5·64	5·70	

In diseased tubers a very acid region was observed around the affected part. In mild forms the disease did not seem to affect the rest of the tuber but in severe cases a slight increase in acidity was noted throughout the tuber. The slight decrease in acidity due to Leaf Roll and the large increase due to Wart Disease confirm results reported by Boas [1919] and Weiss and Harvey [1921] respectively. Severe forms of mosaic, such as crinkle, gave rise to an increase in acidity.

(*d*) *Storage.* A number of tubers of each of six varieties was stored in three different ways, *viz.*: (1) in a pit in the open, (2) in a cool, well-ventilated storehouse, (3) in a refrigerator maintained at 2°. After a few months the average p_H values were as follows:

Original value (October)	5·76
After storage (2)	5·84
,, (1) immediately	5·49
,, (1) after 24 hours at room temperature	5·88
,, (3) immediately	5·39
,, (3) after 24 hours at room temperature	5·83

The tubers from the pit and refrigerator were more acid than before storage, but, on being allowed to stand at room temperature for a day, acquired a p_H slightly higher than the original. This was probably due to increased respiratory activity of the tissue immediately after removal from such storage conditions.

Summary.

The hydrogen and quinhydrone electrodes have been used for p_H measurements of the pulp of the potato (*Solanum tuberosum*), and a micro-electrode enabled measurements to be made at different points of the tissue.

The acidity is not uniform throughout the tuber but depends upon the function of the different parts at different periods in the life of the plant. In the early stages of development the underground stem and the heel end of the tuber are most acid and the average acidity of the tuber increases with maturity. In the dormant state the acidity does not vary much in different parts of the tuber but when sprouting begins the active eyes are most acid.

The acidity of the tuber is independent of environment and is not influenced by large variations in soil acidity.

The differences in acidity due to variety and storage are not significant.

Comparatively large changes in acidity are associated with certain diseases.

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THE INFLUENCE OF THE PLANT UPON SEASONAL CHANGES IN SOIL ACIDITY.

By A. M. SMITH and I. M. ROBERTSON.

(*Edinburgh and East of Scotland College of Agriculture, Edinburgh.*)

(With Four Text-figures.)

The *p*H value of a soil is not constant, and fluctuations which have been reported (3, 9, 15) are probably related to the seasonal variations found in the concentration of electrolytes in the soil (4, 5, 7, 14). For example, the *p*H value usually decreases during summer or during drought, at which times the concentration of electrolytes increases, especially under fallow conditions. It is to be expected, therefore, that the growing plant will affect the acidity of the soil, and this paper is a report of results which demonstrate that point for the potato (*Solanum tuberosum*) and a variety of soils.

Experimental.

The work is considered under the three headings, incubation, pot and field experiments.

Determination of p*H*. The soils were usually examined soon after sampling, by means of the quinhydrone electrode, the procedure being essentially the same as that recently recommended (12). A number of "10 second values" (12) showed that there was no drift due to the presence of manganese dioxide in any of the soils under investigation.

Incubation experiments.

Methods. The following soils were passed through a 2 mm. sieve for these experiments.

Soil B. The mechanical analysis of this soil by the International Pipette Method (2) gave the following percentage composition: coarse sand 20, fine sand 26, silt 24, clay 25, air dry moisture 3·7, loss on ignition 7·4.

Soil P. The sample was taken in March from an uncultivated peaty layer 9–10 in. deep overlying well weathered basic andesite. The loss on ignition of the oven-dry material was 48 per cent.

Soil W. A light sandy soil taken from a mound of glacial sand, which

has not been cultivated for at least 60 years. The loss on ignition, due largely to partially decayed organic matter, was 9 per cent., whilst coarse sand amounted to 50 per cent., and fine sand to 20 per cent.

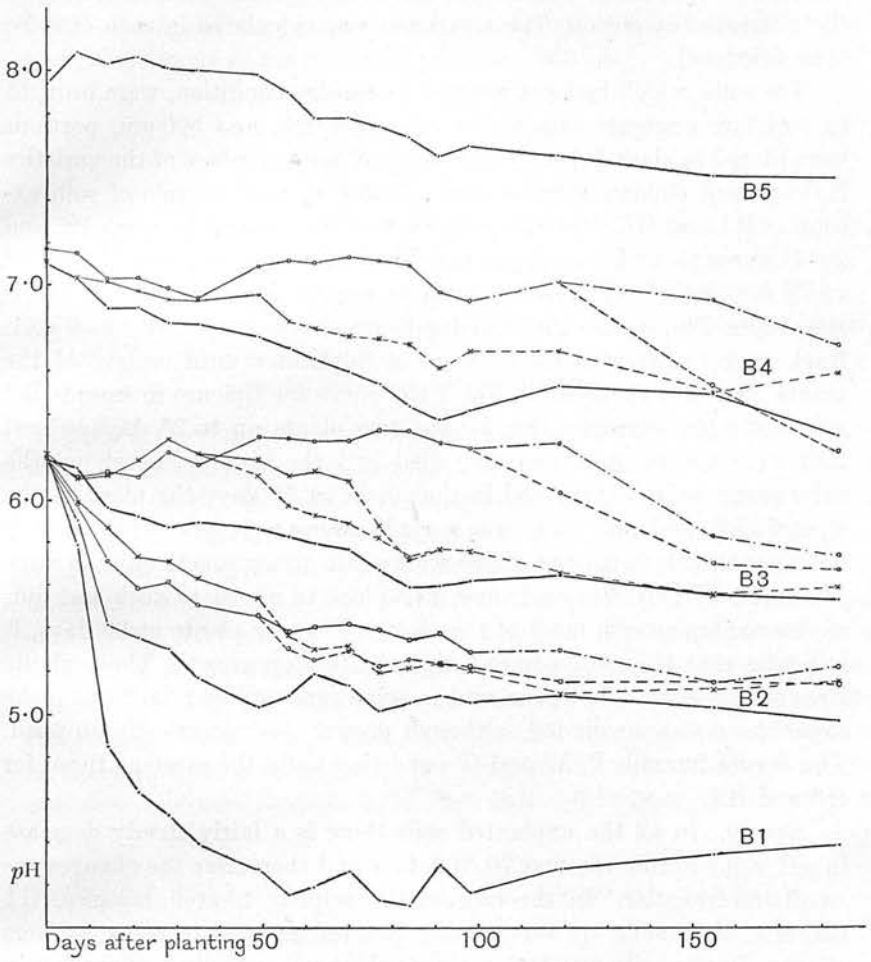

Fig. 1. —•— Fallow —o— Golden Wonder —×— Epicure.

Soil G. A sandy soil, from an uncultivated raised beach close to the south shore of the Firth of Forth, having 68 per cent. coarse sand, 10 per cent. fine sand, 8·5 per cent. loss on ignition.

In addition, samples of B, which had an initial *p*H value of about 6·2, were treated with 0·35 and 0·12 per cent. calcium hydroxide and with 0·024 and 0·12 per cent. sulphur to obtain a range of *p*H values

from about 8 to 4. Similarly, samples of P, having an initial *p*H value of 3·6, were treated with 1·0 and 2·0 per cent. calcium hydroxide to obtain *p*H values of about 5 and 6. Soils W and G, of which the original *p*H values were about 4·5 and 6·7 respectively, were examined only in the untreated condition. The treatment was calculated in each case for oven-dried soil.

The soils, which had not reached an air-dry condition, were brought to moisture contents suitable for plant growth, and 500 gm. portions were placed in glass dishes. Small pieces of potato tubers of the varieties Epicure and Golden Wonder were planted in each sample of soil, excepting B 1 and B 5. The temperature was maintained between 18° and 20° C. throughout the experiments. The dishes were watered daily and small samples of soil were removed at regular intervals for *p*H determinations. The values obtained for B are shown graphically in Fig. 1. Each curve represents the average of duplicates until certain of the plants died. For example, in Fig. 1 the curve for Epicure in sample B 3 represents the average value for the two plants up to 35 days; about that time the plant in one dish died and the broken line shows the subsequent value for the soil in that dish: at 70 days the plant in the second dish died and there was a rapid decrease in *p*H value until at 90 days the soils in the two dishes were again giving practically the same *p*H values. Where the solid lines give place to alternate dash and dot, as, for example, with most of the Golden Wonder plants at 90 days, it indicates that the plants were "nipped" to stop growth. Those plants were very vigorous, however, and in some cases still survived when the experiment was concluded, although growth had practically stopped. The curves for soils P, W and G were essentially the same as those for B 3 and B 4.

Results. In all the unplanted soils there is a fairly steady decrease in *p*H value during the first 70–100 days and thereafter the changes are small and irregular. In the case of the sulphur treated samples, B 1 and B 2, the results are very similar to those reported by other workers (1, 6, 8). In the soils supporting plants there was usually a slight fall in *p*H value until the shoots were established, and then a rise during the rapid stages of growth (except in the case of B 2) to a point higher than the initial value. The *p*H value then decreased, fairly rapidly after the death of a plant, until it approached the value for the unplanted soil. That B 2 should prove an exception was apparently due to the fact that the normal influence of the growing plant in preventing an increase in acidity was more than counteracted by the oxidation of the sulphur in

increasing the acidity. Evidence in support of that explanation is supplied first of all by the fact that the sulphur produced a greater increase in acidity in the fallow than in the planted soil, and secondly by the results (Fig. 2) obtained in an experiment with a portion of soil B which had been treated with an amount of dilute sulphuric acid equivalent to an addition of 0·02 per cent. sulphur.

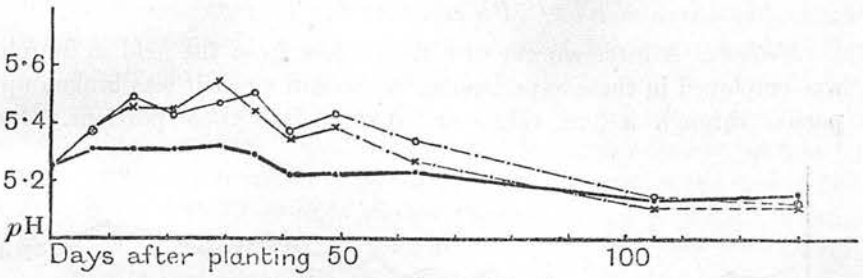

Fig. 2. —·— Fallow —o— Golden Wonder —×— Epicure.

The curves obtained are quite similar to those for B 2 after 70–80 days when the oxidation of sulphur was apparently complete. The decrease in acidity due to the rapidly growing plant and the subsequent return, on "nipping," to that of the unplanted soil are very well marked.

In order to make sure that the differences in *p*H values between unplanted and planted soils were not due to differences in moisture content effected by the growing plant, a number of soils were maintained at definite moisture contents, by daily watering, for a period of 30 days. Measurements of *p*H were made at intervals and the final values are reported in Table I. The results show that different amounts of moisture in the soil, over a range of 10 per cent. in the neighbourhood of the optimum moisture content, have very little effect upon the changes in acidity.

Table I. p*H values after* 30 *days at constant moisture content.*

Soil	B + 0·025 % $S(H_2SO_4)$			B			B + 0·125 % $Ca(OH)_2$		
% moisture	15	20	25	15	20	25	15	20	25
*p*H	5·23	5·25	5·28	5·89	5·85	5·84	6·86	6·87	6·90

Soil	W			G		
% moisture	10	15	20	10	15	20
*p*H	4·42	4·37	4·38	6·24	6·28	6·31

It has been shown (13) that a large increase in the quantity of electrolytes in a soil may occur if it is maintained under conditions suitable for

biological activity. Such an increase modified by the absorption of salts by the plant would account for the results obtained for the fallow soils and the early observations for the planted soils. A return of electrolytes from the decaying plant to the soil is a possible explanation for the final observations for the planted soils, but this point requires further investigation.

Pot experiments.

Methods. A large sample of soil B, taken from the field in March, was employed in these experiments. While still moist it was broken up, passed through a $\frac{1}{4}$ in. riddle and divided into three portions. One

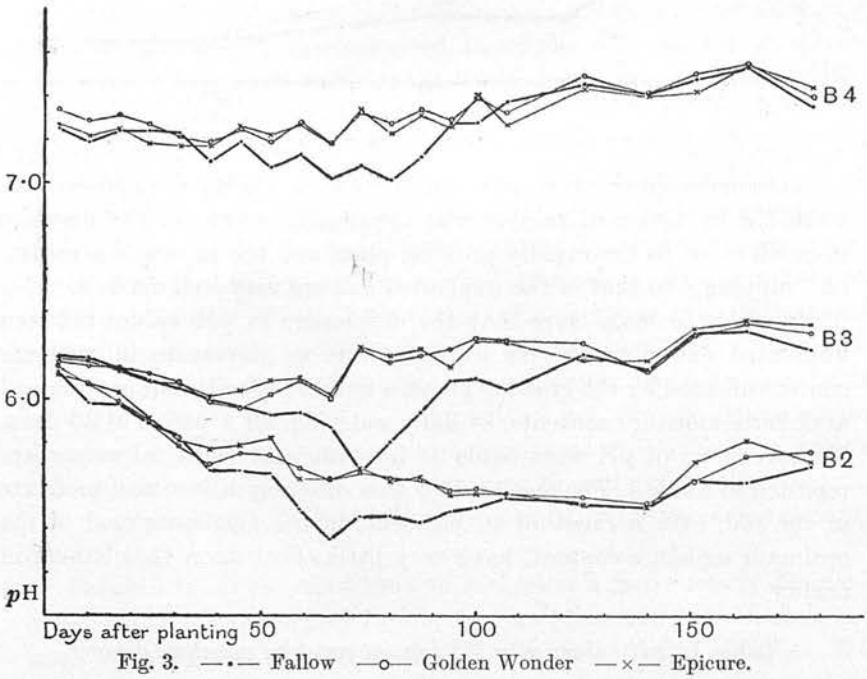

Fig. 3. —•— Fallow —o— Golden Wonder —×— Epicure.

portion (B 2) received 0·024 per cent. sulphur, the second (B 3) was untreated, and the third (B 4) received 0·12 per cent. calcium hydroxide. The additions were calculated in terms of oven-dry soil. Ten pots (12 in. diameter) were filled from each portion and potatoes were planted on April 14. Four pots of B 2 were planted with the variety Epicure, four with Golden Wonder, and two pots were kept fallow. The same was done for the series B 3 and B 4. Samples of soil were taken at weekly intervals until August, and then less frequently until October, by means

of a small auger which reached to the bottom of the pots. During the warm, dry weather of May and June the pots had to be watered frequently, and that probably accounts for the fluctuations and total changes in acidity being small compared with those found in the field experiments described later. The results are shown graphically in Fig. 3.

Results. Considering the fallow pots first, it will be observed that the *p*H value of each soil decreased regularly to a minimum which, except for B 4, existed only for a brief period. Those minima were reached after practically the same length of time as in the incubation experiments, but the total changes in acidity were not so great in this case. The *p*H value then increased, rapidly at first and then more slowly, so that by October 9 it had returned to the original value except in the case of B 2. During the first 30–40 days the soils of the planted pots did not differ materially as regards acidity from those of the fallow pots. The Epicure plants appeared above the surface of the soil in the period 30–40 days, and the Golden Wonder in the period 40–50 days after planting. (The curves for the late variety, Golden Wonder, will be observed to lag somewhat behind those for the early maturing variety, Epicure.) From the time when the shoots appeared until about 60 or 70 days later when the haulms were withering, the *p*H values of the planted soils were definitely higher than those of the corresponding fallow soils. In all three series there may be observed a slight increase in *p*H value when the shoots appeared, which is interesting in view of the similar observation in the case of the incubation experiments. The difference in acidity between planted and fallow soils increased until the plants were flowering, which corresponded approximately with the period of minimum *p*H values for the fallow soils: the difference then became less until eventually the curves for planted and fallow soils were practically the same.

Field experiments.

(*a*) *Plot experiment.* A small area at the College Experimental Farm, Boghall (soil B), was divided into five plots which received the following treatments per acre: plots B 1 and B 2, 1200 and 400 lb. sulphur respectively, B 3 no treatment, B 4 and B 5, 1400 and 4200 lb. calcium hydroxide respectively. The calcium hydroxide was applied in November, 1929, the flowers of sulphur at the beginning of April, 1930. The dressings were approximately the same as those in the incubation and pot experiments already described. At the beginning of May, part of each plot was planted with potatoes. At the end of June, four plants were marked in each plot and thereafter soil samples were always taken from the roots

of each marked plant and from the middle of each fallow area. The first plants appeared at 25 days, the flowers and small tubers had formed at 70 days, the crop was harvested about 150 days after planting.

Results. The fluctuations and total changes in acidity were very great, but the remarkable parallelism amongst the results for all plots suggested the probability of a common factor and some justification for simplifying the presentation of the data by averaging the results for the five fallow and those for the five planted areas. The "average" curves are presented in Fig. 4.

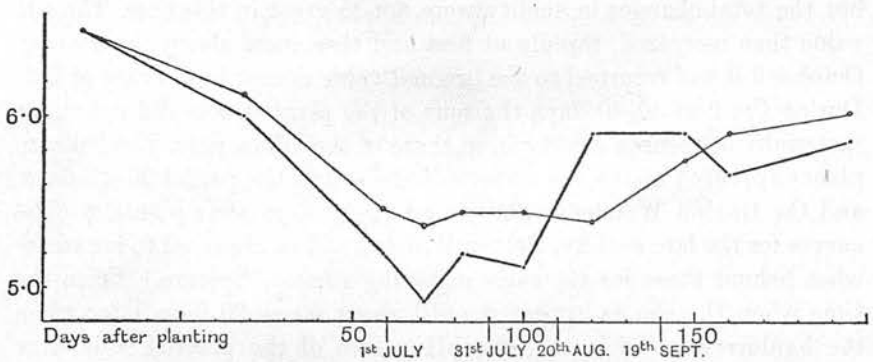

Fig. 4. —•— Fallow —o— Planted.

As in the case of the pot experiments, the curves gradually diverge until about 70 days after planting, at which point the *p*H value of the fallow soils had reached a minimum. The *p*H value of the planted soils remained practically constant during the next 50 days and then gradually increased. The value for the fallow soils, on the other hand, fluctuated considerably, but at 160–190 days had approached close to that for the planted soils.

(*b*) *Field experiments.* Eight soils, within a small area of about 6 miles radius but showing considerable variation in their chief physical and chemical properties, were studied under field conditions in 1929 and 1930. The manurial treatment was similar in all cases, and soil samples were taken as described under the preceding section. The first samples were not taken, however, until the end of June when the crop was fairly well advanced, and a consideration of Fig. 4 shows that a large increase in acidity had taken place by that date at a comparatively "late" locality. Consequently, the first results recorded for the field soils were probably not far removed from the minimum *p*H values for the season. The fluctuations recorded in the later part of the growing

season when, generally speaking, the reaction of the soil is tending to become less acid, seemed to be related to the changes in temperature and moisture conditions rather than to stage of plant growth.

Results. A comparison of the data for the two seasons is limited to a few outstanding features.

In 1929. (*a*) From July 29 to August 18—moderate temperature and high rainfall—there was an increase or little change in *p*H values. (*b*) From August 18 to September 20—high temperatures and moderate rainfall—there was a decrease in *p*H value for the more acid soils and an increase in *p*H value for the less acid soils. (*c*) The final readings, made at the beginning of October, after a spell of cool, wet weather, showed a general increase in *p*H values.

In 1930. (*a*) From July 12 to 23—normal temperature and high rainfall—there was an increase in *p*H values, with two exceptions, for both planted and fallow soils. The plants were not so far advanced as in the similar period (*a*) in 1929, which probably accounts for the more definite change. (*b*) From August 10 to September 2—high temperatures and high rainfall—the planted soils showed somewhat similar tendencies in acidity changes as in (*b*) 1929, whereas all the fallow soils showed a very marked increase in *p*H. In every case the fallow soils became less acid than the planted soils in this period (cp. Fig. 4). (*c*) The final readings, made in October, after a period of cool, wet weather, showed an increase in the *p*H values of planted and fallow soils.

The maximum change for the planted soils varied from 0·1 to 1·4 *p*H units and, generally speaking, the observations on the amount and irregularity of the variations for different soils agreed with reports by other workers (3, 9, 10, 11). None of the soils considered (except plots B 4 and B 5) had received lime for a number of years; the crop was the same in all cases and the manuring was similar: for each year the variation in climate can be regarded as negligible over such a small area: consequently the irregularity of the acidity changes may be ascribed to soil characteristics. In this respect it is noteworthy that at one locality the average *p*H value of the samples increased from 6·2 to 6·9 in 1929 and from 6·4 to 7·0 in 1930.

CONCLUSIONS.

In the early part of the season, when an accumulation of salts takes place (4, 5, 14) on account of the general rise in temperature, the acidity of the soil increases irrespective of climatic factors. Where plants are growing, that accumulation is not so great and the increase in acidity is

not so marked. Towards the end of the growing season, climatic factors, and particularly rainfall, appear to be responsible for irregular fluctuations, but there is a general tendency for the acidity to decrease. Finally, the *p*H value, like the concentration of salts (7), becomes approximately the same for both planted and fallow soils and not far removed from that at the beginning of the season.

It seems, therefore, that variations in soil acidity are definitely connected with changes in the quantities of electrolytes present, and that the effect of the plant is due, at least in part, to absorption of salts. It is possible, however, that bicarbonates formed as a result of plant growth also exert some influence.

Summary.

1. A large number of observations on different soils in incubation, pot and field experiments have been made to determine the effect of the growing plant upon seasonal changes in soil acidity.

2. In every case it has been shown that the acidity of the uncropped soil increases to a maximum during the growing season and that the change may amount to more than one *p*H unit.

3. The plant reduces the change in acidity to a marked extent and at the height of growth there is a considerable difference between the acidity of the cropped and uncropped soil.

4. At the end of the growing season that difference has practically disappeared and the acidity of the soil approaches the value found at the beginning of the season.

5. Irregular fluctuations are related to climatic factors.

6. Attention has been directed to the analogous variations found in the concentration of electrolytes in the soil.

The authors are grateful to Mr A. Robertson for assistance in the later stages of the investigation.

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8.

Separatum

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Recherches sur le Sol

Vol./Bd. III (1932) No 1

The estimation of the buffer capacity of acid soils

(Über die Pufferwirkung des Bodens. — Sur le pouvoir tampon du sol)

By **A. M. Smith** and **R. Coull**
Edinburgh and East of Scotland College of Agriculture

One of the difficulties encountered in estimating the buffer capacity of the soil by the Tovborg Jensen method (4) lies in the fact that a saturated solution of calcium hydroxide is only about $\frac{n}{25}$. Consequently, it is not possible to raise the pH values of some strongly acid soils to 7 or over 7 unless the ratio soil: solution is 1 : 10 or even wider. This is not quite satisfactory since the influence of the soil: water ratio on pH measurements is very uncertain (3) and most investigators now seem to follow the recommendations of the Committee on Soil Reaction Measurements (1) whose work was carried out with the ratio 1 : 2.5 for soil : water.

The difficulty may be overcome by adding solid calcium oxide to the aqueous soil suspension, in place of the dilute alkaline solution, but that method is laborious for routine work on account of the large number of accurate weighings required. A much more concentrated solution of barium hydroxide is readily obtained and might be used, but barium does not effect the same cation exchange in the soil as calcium and the chief value of estimations of buffer capacity is in the study of "lime requirements". Calcium oxide is much more soluble in sucrose solutions than in water, and the object of this note is to show that a sucrose solution of $Ca(OH)_2$ offers a simple method of obtaining a good range of pH values for well buffered and intensely acid soils even with the ratio 1 : 2.5 for soil : solution.

It was found that $\frac{n}{10}$ $Ca(OH)_2$ could easily be obtained in 2 per cent sucrose solutions and that the presence of the sugar had no effect upon the final pH value of the soil suspension if a little chloroform were added to inhibit biological decomposition. The technique employed was essentially the same as that described by Tovborg Jensen. Forty g. samples of air dry soil, passing a 2 mm. sieve, were placed in a series of conical flasks. In the case of peat soils, the moist material, corre-

sponding to 20 g. air dry peat, was taken. Varying quantities of the $\frac{n}{10}$ $Ca(OH)_2$ solution, made up to 100 cc. in each case with 2 per cent sucrose solution, were then added, together with a drop of chloroform. The time required by the soil and calcium hydroxide to reach equilibrium is indefinite and varies considerably for different soils, so that for routine purposes it is necessary to select an arbitrary time

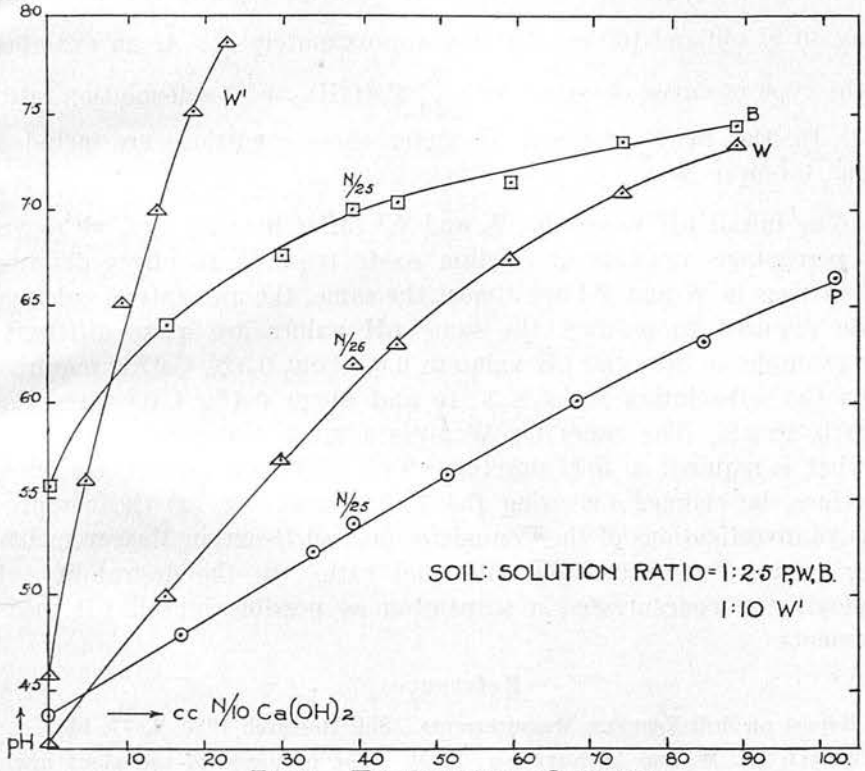

FIG. 1. TITRATION CURVES

of contact. Preliminary work showed that overnight shaking (17 to 18 hours) was suitable and gave reproducible results. After shaking, the flasks were connected in series and a current of carbon dioxide was passed through the suspensions until any excess calcium hydroxide was converted into carbonate; a current of air was then drawn through the flasks until excess carbon dioxide was expelled. The pH values of the suspensions were then determined by means of a quinhydrone electrode.

Typical curves for three soils are given in fig. 1. Soil B is a fertile clay loam, W is an infertile, sandy loam, P is an uncultivated peaty soil having a loss on ignition of 50 per cent. They have already been fully described by Smith and Robertson (2). It will be observed that had the usual $\frac{n}{30}$ $Ca(OH)_2$ been employed with the same soil-solution ratio, only very short portions of the curves would have been obtained. The points marked $\frac{"n"}{25}$ were actually obtained by experiment using 40 g. soil and 100 cc. $Ca(OH)_2$ approximately $\frac{n}{25}$. As an example of the type of curve obtained with $\frac{n}{30}$ $Ca(OH)_2$ and a soil-solution ratio of 1 : 10, the results for soil W under those conditions are included in fig. 1 (curve W^1).

The initial pH values for W and W^1 differ by 0.35 and, although the percentage amounts of calcium oxide required to effect definite pH changes in W and W^1 are almost the same, the amounts of calcium oxide required to produce the same pH values are quite different. For example, to raise the pH value to 6.6, about 0.3 % CaO is required when the soil-solution ratio is 1 : 10 and about 0.4 % CaO when the ratio is 1 : 2.5. The curve for W gives a much closer approximation to what is required in field practice. Two important advantages may, therefore, be claimed for using the 1 : 2.5 ratio, viz. (a) the comprehensive investigations of the Committee on Soil Reaction Measurements were carried out exclusively with that ratio, (b) the desirability of employing as concentrated a suspension as possible in soil pH measurements.

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A CRITICISM OF THE OFFICIAL METHOD FOR THE ESTIMATION OF CALCIUM OXIDE FOR AGRICULTURAL PURPOSES*

BY ALEXANDER LAUDER AND A. M. SMITH
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The object of this note is to draw attention to certain facts which seem to merit discussion on account of their bearing upon the evaluation of lime for agricultural purposes.

The question deals with the by-product of a paper-making firm in the East of Scotland. This firm employs the soda process in the manufacture of a high-grade paper; that is to say, esparto and other cellulose-rich materials are digested with caustic soda (about 6 per cent. solution) and the residual liquor from the pulp is evaporated to dryness and ignited to give black soda ash consisting mainly of Na_2CO_3 together with a certain amount of SiO_2 and impurities from the original fibre. The black ash is usually treated with good quality lime to obtain a fresh supply of NaOH and the by-product is a sludge of $CaCO_3$ which, until a few years ago, went to augment a waste heap. (This sludge is unsuitable for the farmer on account of its physical condition.) This particular firm now burns the sludge to recover lime for causticizing the black ash, but such a cycle of operations cannot be carried on indefinitely because the silica from the esparto gradually finds its way into the sludge.† This decreases the causticizing value of the recovered lime and so a definite proportion has to be discarded and replaced by fresh lime. The amount discarded amounts to 800–1000 tons per annum, and contains about 10 per cent. SiO_2; it is with the agricultural value of this product that we have been concerned.

The official method of assessing a burnt lime is to determine the percentage CaO which may be extracted by a 10 per cent. sugar solution; (there is some doubt as to whether CaO forms definite compounds with sucrose, but that need not be considered at present) and the figure obtained for this by-product is only about 60 per cent. On the other hand its causticizing value, found both in practice and as estimated by a modification of the American official method for available CaO, is equivalent to 75–80 per cent. CaO. Further, when estimated by the decomposition of $(NH_4)_2SO_4$ a figure of over 80 per cent. for CaO is obtained. The total CaO present is about 80 per cent. and there is 1–2 per cent. alkali. It

* This paper was read before the Chemistry Committee in December 1931, and was unfortunately omitted from Vol. IX.

† It is chiefly the esparto which is responsible for the silica.

seems, therefore, that there is about 15–20 per cent. CaO which is combined with SiO_2 but only in some loose form of combination, because it may readily be converted into carbonate by means of CO_2.

There has been no opportunity of carrying out field experiments to test the material, and it is obvious that little would be gained without an elaborate series of plots studied over a number of years. A considerable number of laboratory tests have been made, however, with a variety of soils. The simplest and most direct method appeared to be to measure the effects of the product in reducing soil acidity and to compare the results with those obtained with a lime of definite purity. Usually 20-gm. samples of soil were placed in a series of flasks with 100 c.c. CO_2-free water, and a range of quantities of lime was added to the flasks, which were then shaken overnight. Carbon dioxide was bubbled through the flasks until any excess CaO was converted to $CaCO_3$, and then air until excess CO_2 was expelled. The *p*H values of the suspensions were determined electrometrically and a curve prepared showing the relationship between acidity and amount of lime added.

Under these conditions, the availability of this product depends upon the type of soil employed, because the amount of lime required to effect similar changes in *p*H necessarily varies with the original acidity of the soil and its buffer capacity. Up to a certain point on the curves, however, values corresponding to about 80 per cent. CaO have been obtained. After that point, which is always beyond what would be attempted in actual liming practice, there is a progressive decrease in the apparent available CaO.

Generally speaking, the results support all the other figures except that obtained by sugar extraction, and indicate that the official method does not give an accurate measure of the value of the lime for reducing soil acidity. A difference of 15 per cent. CaO is of considerable importance, and it seemed desirable to submit the question for discussion before this Committee, the members of which are intimately concerned with the application of the Regulations of the Fertilizers and Feeding Stuffs Act, 1928.

ANALYSIS OF BURNT LIME.

	Equivalent CaO per cent.	
	By-product Lime.	*Standard Lime.*
Official Method	63·5	94·3
Soil Method .. .. .. about	80	100
Distillation $(NH_4)_2SO_4$	80·5	95·3
Total Bases (phenolphthalein) ..	81·9	—
Total Ca as CaO	80·0	—
Insoluble in acids .. .. about	10%	—

Reprint from the Transactions of the Second Commission and Alkali-Subcommission of the International Society of Soil Science. Kjöbenhavn (Danmark). 1933. Vol. A. pp. 107—108.

The variation in soil acidity.

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As a result of about 3000 determinations of acidity made in the course of recent years, it has become increasingly evident that the pH value of an acid soil is liable to fluctuate to a marked extent. The measurements have invariably been made according to the recommendations of the Second Commission of the International Society of Soil Science[1] on a number of soil types varying considerably in their principal chemical and physical properties. The numbers of observations on samples taken carefully from pots and plots as well as from fields have been large enough to make sampling errors small compared with the larger fluctuations: even the differences in pH between the moist soil and the air dry soil have been found to be small in comparison with the differences obtained at different times of sampling.

Generally speaking, in the East of Scotland area (the mean summer temperature is 12·2 C°, the mean winter temperature is 4·2 C° and the rainfall of 622 mm is fairly evenly distributed throughout the year) the pH value usually falls during the growing season and then tends to rise again. Rapid changes in climatic conditions such as short periods of drought or heavy rain, or abnormal temperatures may on occasion, however, upset the general tendencies so that it is not possible to describe

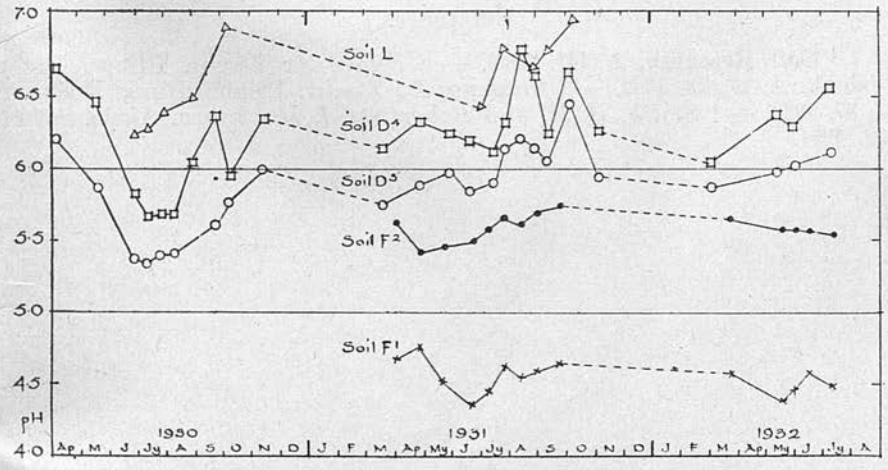

Fig. 1. Seasonal Variation in SOIL ACIDITY

the variations in definite terms. Most of the observations have been made between March and October. Since the soils are generally saturated with moisture during the winter months and are seldom frozen for long periods, it may be assumed that any changes in pH are small compared with those in summer.

A few typical cases are shown in the accompanying diagram (fig. 1) to indicate the nature and extent of the changes which have been observed. The fluctuations vary from soil to soil and in some cases exceed one pH-unit during the season: they are much greater on unplanted soil than on the same soil carrying a crop:[4] the more important changes are periodic and are closely related to the concentration of electrolytes in the soil; they are not materially reduced by making the pH measurements in N . KCl suspensions.

Several investigators[2] [3] have recently submitted similar figures, and there would seem to be no doubt that a single determination of soil acidity may be very misleading. The possible effect of soluble salts on the pH of a soil suspension in water has long been recognised, and the use of a KCl-suspension has been advocated to surmount the difficulty of seasonal variation in the concentration of the soil solution. It has been found, however, that the addition of potassium chloride to the suspension may only serve to bring about a fairly uniform decrease in the pH values of different samples without eliminating the seasonal fluctuations. On the other hand, a preliminary washing of different samples until the filtrate attains a comparatively low specific resistance tends to raise the pH values to a more constant figure. It is suggested that such a preliminary treatment is desirable for single samples taken during the growing season. The bulk of the soluble salts, which tend to accumulate in warm dry weather, are thus removed and cannot give rise to an indefinite amount of exchange acidity. It is obvious, however, that the question of soil acidity, both direct or indirect, still presents some difficulties which call for further study now that the method of making the measurement seems to have been satisfactorily standardised.

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[1] Soil Research. 2. 141. 1930. — [2] *Kühn, S.:* Ztschr. Pflanz. Düng. Bodenk. A. 27. 73. 1932. — [3] *Pozdena, L.:* Ztschr. Pflanz. Düng. Bodk. A. 27. 87. 1932. — [4] *Smith, A. M.* and *Robertson, I. M.:* Journ. Agric. Sci 21. 822. 1931.

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